Novel homozygous *PANK2* mutation identified in a consanguineous Chinese pedigree with pantothenate kinase-associated neurodegeneration

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Abstract. Pantothenate kinase-associated neurodegeneration (PKAN) is a rare autosomal recessive neurodegenerative disorder resulting from pantothenate kinase 2 (*PANK2*) gene mutations. It is clinically characterized by early onset of extrapyramidal symptoms, with or without pigmentary retinopathy, optic atrophy and acanthocytosis. The specific radiographic appearance of PKAN is the eye-of-the-tiger sign. However, there are few studies regarding PKAN patients of Chinese Han ancestry. In the present study, a Chinese 20-year-old female with an 8-year history of unsteady walking and involuntary movements is described. Brain magnetic resonance imaging revealed eye-of-the-tiger sign. Following sequencing of *PANK2*, a novel homozygous c.863C>T (p.P288L) mutation was identified in the patient and heterozygous c.863C>T was identified in her consanguineous parents. The absence of this mutation in the 1000 Genomes database, The Exome Aggregation Consortium, and 200 controls demonstrated that this mutation was probably pathogenic for PKAN in this family. In addition, the *PANK2* c.863C>T mutation was predicted to be deleterious by SIFT, disease causing by Mutation Taster and probably damaging by PolyPhen2.

Introduction

Pantothenate kinase-associated neurodegeneration (PKAN), also termed Hallervorden-Spatz syndrome (HSS), is a rare autosomal recessive neurodegenerative disorder resulting from mutations of the pantothenate kinase 2 (*PANK2*) gene (1). It is the most common form of neurodegeneration with brain iron accumulation (NBIA) (2). Clinically, it is characterized by childhood onset of dystonia, dysarthria, rigidity, and choreoathetosis, with or without pigmentary retinopathy, optic atrophy, and acanthocytosis (3). Approximately one-third of the PKAN patients showed cognitive decline or dementia (3). In typical PKAN, symptoms present within the first decade of life and usually rapidly progress, culminating in early mortality. However, in atypical PKAN, the onset of extrapyramidal symptoms is later, and the progression is slower and more variable. The characteristic imaging pattern of PKAN is eye-of-the-tiger sign, which consists of bilateral hyper-intensity within surrounding hypo-intensity in the globus pallidus in T2-weighted images (4).

The prevalence of PKAN is low, particularly in the Chinese population. To the best of our knowledge, no >30 PKAN cases of Chinese ancestry have been documented so far (5-10). The current study describes a PKAN patient of a consanguineous Chinese pedigree who exhibited the novel homozygous *PANK2* mutation.

Materials and methods

Subjects. The current study was approved by the ethics committee of the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China). A single PKAN patient from a consanguineous Chinese pedigree was recruited. Written informed consent was obtained from the participants prior to blood sample collection. In addition, 200 healthy individuals of Chinese ancestry were recruited as control subjects. The neurological examinations (including consciousness, recognition, gait, cranial nerve, motor, sensory, reflex, pathological signs and coordination) and clinical evaluations (including age at onset, symptoms and signs, disease course and treatment) were performed. Brain magnetic resonance imaging (MRI) was conducted in the PKAN patient. Blood samples (3 ml) were collected for laboratory inspection and gene sequencing.

Mutation analysis. After obtaining informed consent, genomic DNA was extracted from 3 ml peripheral blood

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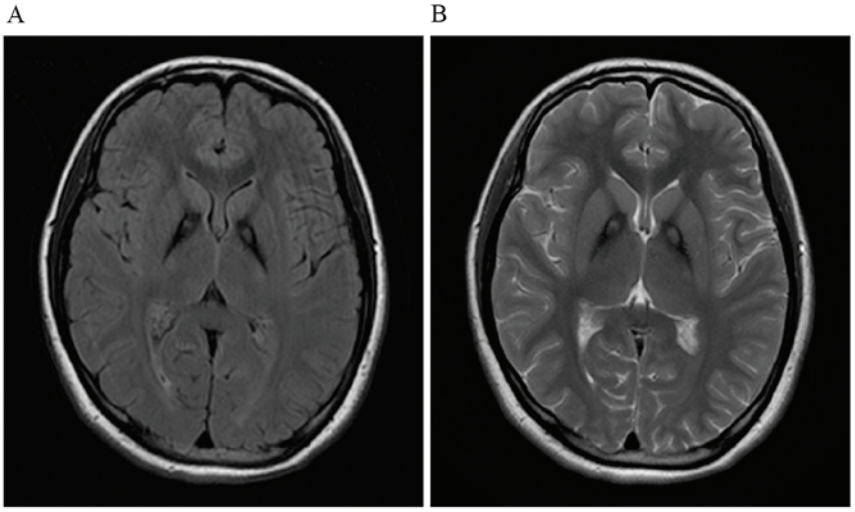

Figure 1. Axial magnetic resonance imaging demonstrates bilateral hyperintense pallidal areas on background areas of T2 shortening, termed eye-of-the-tiger sign. (A) T2-fluid attenuated inversion recovery and (B) T2-weighted.

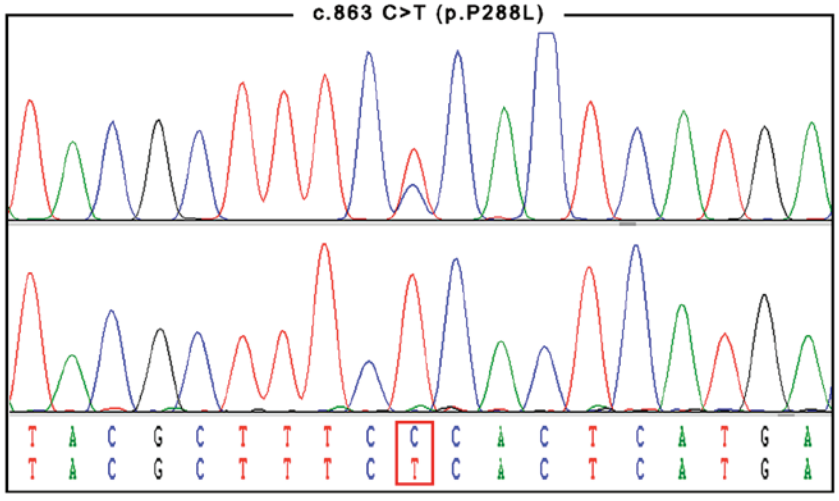

Figure 2. Chromatogram of the novel pantothenate kinase 2 c.863C>T mutation. Upper panel, the heterozygous sequence in the proband's consanguineous parents; lower panel, the homozygous mutation identified in the proband.

using a QIAamp DNA Blood Minikit (Qiagen GmbH, Hilden, Germany). Mutational investigation of *PANK2* gene was performed by polymerase chain reaction (PCR) amplification and direct DNA sequencing, according to a previous study (1). Briefly, forward and reverse primers were designed to amplify each of the seven exons of *PANK2* and all exons of *PANK2* and the respective intron-exon boundaries were amplified by PCR according to a previous study (1). The purified PCR products were sequenced using an ABI 3730 Automated DNA Sequencer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The sequencing results were aligned to the NCBI human reference DNA sequence of *PANK2* (Ensembl gene ID: ENSG00000125779; http://asia.ensembl.org/index.html). Amino acid and nucleotide changes were identified and numbered corresponding to their position within *PANK2* mRNA. SIFT (http://sift.jcvi.org), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org) were used to predict the pathogenicity of the identified mutation.

Results

Clinical features of the patient. The proband was a 20-year-old female who had an 8-year history of gait difficulties and involuntary movements. Her delivery and postnatal period was unremarkable. The patient had normal acquisition of developmental milestones until the age of 12 years when unstable walk and involuntary movements began to develop. Her upper limbs suffered moderate chorea and ballismus, which became accentuated by anxiety and diminished when she was calm. Clonazepam at dose of 0.5 mg was prescribed and the symptoms were significantly relieved. Two years later, difficulties with walking and the involuntary movements were aggravated and the patient began to exhibit dysarthria. The patient experienced did not completely alleviate the symptoms. Oral administration of benzhexol (2 mg, three times per day) was effective in the first two months. When the patient sought medical care in the Department of Neurology, Second Affiliated Hospital, Zhejiang University School of Medicine

(Hangzhou, China) in December 2015, she presented dysarthria, involuntary movements and unsteady gait.

On cranial nerve examination, the extra-ocular movements of the patient were normal without nystagmus and visual field deficits. Choreiform movement was observed in the face. Pronunciation was slurred with mild palilalia, although her tongue and soft palate were at midline. The patient's muscle strength was normal, although muscle tension was decreased in the extremities and trunk. Chorea was obvious in the patient's upper limbs. No sensory abnormalities were observed and tendon reflexes were brisk, while the Babinski sign was bilaterally negative. Finger-nose and heel-knee-shin test results were normal. The patient's gait was unstable although the Romberg's test result was negative. The patient's recognition was not impaired, with a mini-mental state examination score of 29/30. Laboratory findings included normal serum ferritin, ceruloplasmin, albumin and lipoprotein levels. The blood smear was negative for acanthocytes. A brain MRI revealed iron accumulation in the bilateral globus pallidus and putamen (Fig. 1).

The patient's father and mother were consanguineous, healthy and did not exhibit gait difficulty or dysarthria.

Identification of the novel PANK2 mutation. After sequencing of the *PANK2* gene, a homozygous c.863C>T mutation was identified in the proband (Fig. 2). This mutation was located in exon 2 of *PANK2*, causing a proline to leucine substitution at position 288 (p.P288L) of the enzyme. Her consanguineous parents were heterozygous carriers of c.863C>T. The homozygous c.863C>T mutation was absent in the 1000 Genomes database (http://www.1000genomes.org/), The Exome Aggregation Consortium (ExAc; http://exac.broadinstitute.org/) and all 200 control individuals. The homozygous c.863C>T mutation of *PANK2* was predicted to be deleterious by SIFT, disease causing by Mutation Taster and probably damaging by PolyPhen2.

Discussion

PKAN is a neurodegenerative disorder associated with excessive iron deposition in the globus pallidus (11). It was first described by German neuropathologists Hallervorden and Spatz in 1922, therefore is also referred to as HSS (12); however, was subsequently renamed PKAN (13). In the present study, a Chinese 20-year-old female with an 8-year history of unsteady walking and involuntary movements is described. A brain MRI revealed typical eye-of-the-tiger sign. After sequencing the *PANK2*, a homozygous c.863C>T (p.P288L) mutation was identified in her genetic DNA. Further genetic investigations revealed a heterozygous c.863C>T mutation in her consanguineous parents. To the best of our knowledge, this mutation has not previously been reported; therefore it is considered to be a novel mutation. The absence of this mutation in the 1000 Genome database, ExAC, and 200 control individuals demonstrated that this mutation was potentially pathogenic for PKAN in this particular family. In addition, it was predicted to be deleterious by SIFT and Mutation Taster.

The patient described in the current study experienced early onset and presented with an unsteady walk, choreic movements, and dysarthria as primary symptoms. The cognitive impairment was not obvious, although the patient had experienced the disease for 8 years. These observations were consistent with two existing reports of Chinese PKAN patients (5,6). Notably, cognitive impairment was not prominent in Asian PKAN patients (2,14,15). By contrast, the prevalence of pyramidal signs, mental impairment, and Parkinsonism was higher in Caucasian patients (16). This discrepancy is potentially due to the ethnic differences.

To date, >100 mutations within PANK2 have been reported in PKAN cases worldwide (http://www.hgmd.org). The majority of *PANK2* mutations are missense mutations, while duplication, deletion, splice-site mutation and exon deletion have also been reported (16). The majority of previously reported Chinese PKAN patients were detected to be carrying compound heterozygous mutations. In the present study, the patient carried a homozygous *PANK2* mutation. Due to the Chinese single-child policy that commenced in 1980, this patient did not have any siblings. Therefore, co-segregation could not be conducted in this family. However, the early onset of extrapyramidal symptoms, characteristic eye-of-the-tiger sign in MRI, and identification of the homozygous *PANK2* mutation revealed that this was a case of PKAN.

The mechanism by which mutations in the *PANK2* gene lead to iron accumulation in the brain remains to be elucidated. *PANK2* is an essential regulatory enzyme in coenzyme A biosynthesis (13). In addition, the protein encoded by *PANK2* is crucial in the metabolism of pantothenate. A deficiency of *PANK2* is reported to result in the accumulation of cysteine-containing substrates, which may undergo rapid auto-oxidation in the presence of iron, leading to free radical generation and cell damage (1). A limitation of the current study was that functional experiments were not performed to verify the pathogenicity of the identified mutation. Thus, further investigations are required.

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