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Early-onset Parkinson's disease in a Chinese population: ^{99m}Tc-TRODAT-1 SPECT, *Parkin* gene analysis and clinical study

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Abstract

Early Onset Parkinson's Disease (EOPD) is characterized by selective degeneration of nigrostriatal dopaminergic neurons and a marked response to levodopa. However, at present, few methods are available as diagnostic tools for EOPD except for ^{18}F-DOPA PET. In addition, little is known about the correlation between clinical severity, neuroimaging grading and genetic susceptibility. In the present study, ^{99m}Tc-TRODAT-1 SPECT and brain MRI were used to identify 30 cases of non-familial EOPD from a Chinese cohort of 230. All 30 PD patients had an age of onset of less than 55 years (mean age at onset, 41.5 ± 9.3 years). Each of the 30 EOPD cases was sub-classified into one of five stages based on the ^{99m}Tc-TRODAT-1 SPECT findings. In the early stages of PD (stages 1 and 2), a lower uptake of ^{99m}Tc-TRODAT-1 in the putamen was found, while uptake in the caudate nucleus was normal. In the latter stages (stages 3, 4, 5), 24 patients revealed a diffuse and uniform loss of ^{99m}Tc-TRODAT-1 uptake in the putamen and the caudate nucleus. Further, in conventional genetic studies of the 30 patients, six novel mutations were found in the *Parkin* gene, and these included five heterozygous point mutations (C441R, Q311H, V258M, C212G, and S193I) and one homozygous deletion (exon 10-12). Known polymorphisms (Ser167Asn, Val380Leu) were also found in a number of patients. However, gene dosage analysis did not reveal any compound heterozygous mutations in these 30 patients using quantitative duplex PCR. This is the first study to examine EOPD patients of Chinese ethnic background (not exhibiting a definite familial trait), to offer a complete genetic analysis of the *Parkin* gene, and to correlate clinical stages of the disease with dopamine re-uptake.

Keywords: Early-onset Parkinson's disease; *Parkin* gene mutation; SPECT imaging; Dopamine transporter; Chinese population

1. Introduction

Parkinson's disease (PD) is a very common neurological disorder. Clinical symptoms include rigidity, bradykinesia, and resting tremors. The main pathological feature of PD is the degeneration of nigrostriatal dopaminergic neurons. The prevalence of PD is about 2% in persons over 60 years of age [1]. However, PD can also manifest earlier in life, and this is termed early onset PD (EOPD). The age used to define EOPD varies from below 40 years of age [2] to up to 58 years [3]. The primary cause of PD is still unknown. Although most instances of patients with PD are sporadic, a small group of patients have been shown to have familial PD. Thus, extensive genetic analysis of these families has been used to shed light on the pathogenesis of PD [4].

In the last few years, mutations in four human genetic loci have been identified as causing PD, namely *α-synuclein, parkin, tau, UCH-L1*. Mutations in α-synuclein, tau and *UCH-L1* are relatively rare as compared to those in the *Parkin* gene in patients with PD. Point mutations in exon 3 and 4 of *α-synuclein* have been associated with autosomal dominant PD [5,6]. Point mutations of the *tau* gene in exon 9, 10, 12, and 13 have been found in Parkinsonism with

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frontotemporal dementia [7,8]. A point mutation in *UCH-L1* has been detected in a German family with autosomal dominant Parkinsonism [9]. In recent studies, mutations in *NR4A2, PINK-1, DJ-1* and *synphilin* were also shown to be associated with PD [10–13].

The Parkin protein is an E3 ubiquitin-protein ligase with an ubiquitin-like domain at its N-terminus. Parkinsonism associated with mutations in the *Parkin* gene (Park2) (OMIM 602544) is an autosomal recessive juvenile disorder (AR-JP) characterized by the early onset of symptoms, a slow progression, and a high frequency of levodopa-induced dyskinesias [14]. *Parkin* gene mutations in PD patients include exonic deletions, truncation and missense mutations [4,15]. Originally described in Japan [16], EOPD has been identified in other races and geographic regions such as the Jewish Diaspora [17], North Africa, and Europe [3]. As yet, however, there have been no reports of studies of clinical presentation and *Parkin* gene mutations in EOPD in Chinese populations.

An accurate diagnosis of Parkinson's disease is important not only for counselling and management of patients, but also for conducting pharmacological and epidemiological studies. Previous studies have shown that an unexpectedly high rate of misdiagnosis occurs if the diagnosis is based only on clinical diagnostic criteria [18]. In addition, post-mortem studies have shown a close relationship between the concentration of dopamine transporters (DATs) and striatal dopamine levels. Since DATs are located only on dopaminergic nerve terminals and TRODAT-1, a tropane derivative, binds specifically to DATs, ^{99m}Tc-TRODAT-1 SPECT should provide an ideal tool for diagnosing PD [12,19].

In this study, we first correlated ^{99m}Tc-TRODAT-1 SPECT data from PD patients with the clinical presentation of PD. We then performed extensive mutational analyses of *Parkin* gene on 30 cases of EOPD without familial traits that were diagnosed using ^{99m}Tc-TRODAT-1 SPECT.

2. Materials and methods

2.1. Patients

All patients were recruited into the registry between 1998 and 2001, receiving a detailed neurological examination, a medical record review and magnetic resonance imaging (MRI). The diagnosis of PD was defined by clinical research criteria [20] and by a reduction in DATs as indicated by ^{99m}Tc-TRODAT-1 SPECT data. Patients with secondary or symptomatic Parkinsonism, such as PD induced by drugs or environmental toxins, or Parkinson-plus syndromes, such as progressive supranuclear palsy (PSP), or multiple system atrophy (MSA), were excluded from this study. The following data collection methods were applied to each patient: blood sampling, clinical examination, family history and drug usage questionnaires. All relatives of patients were interviewed and examined physically as completely as possible. A genetic consent form for this registry gave permission for blood samples to be used in *Parkin* gene analysis. Information on age-at-onset of PD and medical records were obtained by direct interview. Thirty patients from this registry whose age at onset of PD were younger than 55 (mean age at onset, 41.5 ± 9.3 years) were included in this study. Researchers blind to the *Parkin* mutation status of the patients conducted all clinical data collection.

2.2. ^{99m}Tc-TRODAT-1 SPECT images

Patients in a supine position had their head fixed in a holder. ^{99m}Tc-TRODAT-1 was prepared as previously described [21]. Every subject received a single bolus injection of 740 MBq ^{99m}Tc-TRODAT-1 and 15 dynamic images of the brain were acquired over 30 min. Images were reconstructed using back-projection with a Metz filter. Regions of interest were marked on one side of the striatum as reference for the corresponding MR image and were fitted to the other side. Regions of interest were drawn over the whole striatum, putamen, and caudate nucleus of each hemisphere on composite images of the three slices with the highest TRODAT-1 basal ganglia activity. The occipital cortices (OC) were also drawn in the same way and served as areas from which the background signal could be calculated. The specific uptake of ^{99m}Tc-TRODAT-1 was calculated by subtracting mean counts in the OC from mean counts in the whole striatum, putamen (P), or caudate nucleus (C) and dividing the result by the mean counts from the background, that is, C/OC or P/OC [12].

The images were interpreted both visually and semi-quantitatively (Table 2). We classified these findings with regards to uptake of ^{99m}Tc-TRODAT-1 as follows: Stage 1, diminished uptake over unilateral putamen; Stage 2, diminished uptake over bilateral putamen; Stage 3, diminished uptake over unilateral caudate and bilateral putamen; Stage 4, diminished uptake over bilateral caudate and bilateral putamen; Stage 5, negligible uptake over bilateral caudate and bilateral putamen (Fig. 1).

2.3. Conventional mutational and gene dosage analysis of Parkin gene

Blood samples were collected after informed written consent. High molecular weight genomic DNA was extracted using standard techniques from peripheral blood leucocytes [4]. Genotyping was performed using Polymerase Chain Reaction (PCR). Each of the 12 exons of the *Parkin* gene were amplified separately using oligonucleotide primer pairs, as previously described [14]. The PCR reaction mixture consisted of 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.001% gelatin, 1.5 mM $MgCl_2$, 0.08 mM of each dNTP, 2.5 U of ampliTaq DNA polymerase (Perkin–Elmer, CA), 10 pmol of each primer, and 50 ng of genomic DNA template in a total volume of 25 μl. The PCR conditions