Short Report

Clinical features of Chinese patients with Huntington’s disease carrying CAG repeats beyond 60 within \textit{HTT} gene


Patients with Huntington’s disease (HD) carrying CAG repeats beyond 60 are less frequently seen and clinical features of them have been rarely reported. We identified four unrelated patients carrying CAG repeats beyond 60 (84.0 ± 13.76, ranging from 74 to 104) from 119 Chinese HD patients via direct sequencing. These four were all early onset with a mean age at presenting symptom of 9.8 ± 1.71 years. Paternal transmission was found in three of them and the fourth was apparently sporadic. In addition, they had atypical onset symptoms including epilepsy, intellectual decline, tics and walking instability, which might lead the clinicians to make the wrong diagnosis in the early stage of disease. Our work explores clinical features of Chinese HD patients with an expanded CAG repeat over 60 and may help the clinicians make a correct diagnosis in the early stage of disease.

Conflict of interest

The authors report no conflicts of interest.

Huntington’s disease (HD) is an autosomal dominant inherited neurodegenerative disease. It usually starts insidiously in mid-adult life with characteristic manifestations of progressive motor dysfunction, cognitive impairment and psychological disorders (1). HD is caused by an unstable CAG triplet repeats expansion in exon 1 of \textit{HTT} gene with at least 36 repeats (2, 3). It has been proven by many studies that CAG repeat length is inversely related to age at onset (AAO) (4, 5).

Large CAG expansion over 60 accounts for 4% of the spectrum of \textit{HTT} mutation (6) and HD patients with large expansion (>60 CAG repeats) develop symptoms at an early age, usually with rapid progression and atypical motor disorders other than chorea (7–9). It is reported that the expanded CAG repeat size of juvenile-onset HD (JHD) usually exceeds 60 (10, 11). But it could also be below 59 and even as low as 41 (12–14). Most of the reports focus on JHD and little concerns about the clinical features of...
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Table 1. Initial symptom of Huntington’s disease (HD) patients

<table>
<thead>
<tr>
<th>Initial symptom</th>
<th>Patients with CAG repeats beyond 60 (n = 4)</th>
<th>Patients with CAG repeats below 60 (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizures</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>Intellectual decline</td>
<td>1 (25%)</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Walking instability</td>
<td>1 (25%)</td>
<td>7 (6.1%)</td>
</tr>
<tr>
<td>Tics in limbs</td>
<td>1 (25%)</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Ataxia</td>
<td>0</td>
<td>7 (6.1%)</td>
</tr>
<tr>
<td>Psychiatric disorder</td>
<td>0</td>
<td>4 (3.5%)</td>
</tr>
<tr>
<td>Chorea movement</td>
<td>0</td>
<td>91 (79.2%)</td>
</tr>
<tr>
<td>Memory declined</td>
<td>0</td>
<td>2 (1.7%)</td>
</tr>
</tbody>
</table>

HD patients with a length of CAG repeat over 60, especially in China. To our knowledge, there were only two individual case reports on Chinese HD patients with CAG repeats beyond 60 (15, 16).

Here, we describe the clinical and genetic features of four unrelated patients carrying CAG repeats beyond 60 after investigating 119 HD patients. We attempt to explore clinical features of HD with large expansion (>60 CAG repeats) in Chinese HD patients and help clinicians recognize it in the early stage of the disease.

Materials and methods

Subjects

One hundred and nineteen Chinese Han HD patients from 110 families confirmed by genetic testing were recruited. They were from Southeastern China and enrolled consecutively in Genetic Clinic of Huashan Hospital between February 2008 and October 2012. Their clinical data were investigated retrospectively. The study was approved by the ethics committee of Huashan Hospital. Informed consents were signed by patients or the parents of patients younger than 18 years old.

Genetic analysis of CAG repeats in HTT gene

Genomic DNA was extracted from peripheral ethylene diamine tetraacetic acid (EDTA)-treated blood using Blood Genomic Extraction Kit (Lifefeng Biotechnology Co., Ltd., Shanghai, China). Genetic testing was conducted for all 119 patients, as previously reported (17), and CAG repeat sizes were further determined by direct sequencing.

Results

Genetic analysis of HTT gene

Among 119 HD patients, 4 were found carrying CAG repeats beyond 60, which were estimated by electrophoresis analysis (Fig. S1, Supporting Information) and further confirmed by sequencing (Figs S2–S5). Mean expanded CAG repeat number of these four patients was much larger than that of the other 115 patients (84.0 ± 13.76 vs 45.3 ± 3.64). The repeat sizes for the affected uncle of patient 3 and affected father of patient 4 was 17/53 and 16/45, respectively (Figs S6 and S7), much smaller than that of the proband.

Clinical features

The mean AAO of the four patients with large CAG expansion was much earlier than that of the other 115 patients (9.8 ± 1.71 vs 40.2 ± 10.75 years). The average delayed time from onset to molecular diagnosis in these four patients was 4.0 ± 2.16 years, ranging from 2 to 7 years. In addition, they started with atypical symptoms while the other 115 patients begun with characteristic HD symptoms (Table 1). The characteristics of these four patients were summarized in Table 2.

Patient 1 (Fig. 1; family 1, IV1) started with frequent seizures during sleep time at age 8. He primarily presented with jerking in upper limbs during the ictal period and each episode lasted for 1–2 min. Then, he gradually developed shrug in the right shoulder and exhibited poor attention and impaired intelligence. On neurological examination, the nystagmus was negative. Ankle clonus and Babinski’s sign were positive. Muscle tone of lower limbs and tendon reflexes were increased. Epileptiform discharges were depicted on electroencephalograph. He died at age 11 with an unclear cause. All his family members in four generations were affected except one of his uncles. Their AAO ranged from 30 to 40 years and the average disease course was about 10 years. Of all the affected family members, only his younger aunt was still alive with a disease course of 2 years.

Patient 2 (Fig. 1; family 2, III1) started with tics in limbs at age 10. The tics were always quick and jerky just like the typical tics manifestation in

Table 2. Characteristics of four patients carrying CAG repeats beyond 60

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset (years)/gender</td>
<td>8/male</td>
<td>10/male</td>
<td>9/male</td>
<td>12/female</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>10</td>
<td>13</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Onset symptom</td>
<td>Seizures</td>
<td>Tics in limbs</td>
<td>Intellectual decline</td>
<td>Walking instability</td>
</tr>
<tr>
<td>Number of CAG repeats</td>
<td>17/104</td>
<td>18/82</td>
<td>17/76</td>
<td>17/74</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Paternal</td>
<td>No family history</td>
<td>Paternal</td>
<td>Paternal</td>
</tr>
</tbody>
</table>
Huntington’s disease patients with large CAG repeat expansion

Fig. 1. Pedigrees of four Huntington’s disease patients carrying CAG repeats beyond 60. The arrows indicate the probands.

Tourette’s syndrome. He experienced gait disorder and slurred speech and gradually developed involuntary nictation and wiggle of head, which existed even during sleep. Foot dystonia was observed and seemed like ‘Pes adductus’. Neurological examination revealed increased muscle tone. No family history was reported. The request of genetic testing to his parents was declined.

Patient 3 (Fig. 1; family 3, III1) started with intellectual decline at age 9. Three years later, he complained clumsiness in the left hand and gradually exhibited dysarthria and drop attacks. He was incommunicative. On neurological examination, nystagmus was negative. Tendon reflexes were brisk and Babinski’s sign was positive. His father and three uncles were all affected with AAO of 20–35 years. His father and grandfather committed suicide at age 37 and 50, respectively.

Patient 4 (Fig. 1; family 4, III1) started with walking instability and gradually exhibited bradykinesia, dysphagia and speech with a lisp. She developed a kyphosis due to the severe trunk dystonia. On neurological examination, ataxia signs were evident. Muscle tone and tendon reflexes were all increased. Ankle clonus was positive. Slow saccades were observed and nystagmus was negative. She was initially clinically diagnosed as spinocerebellar ataxia (SCA). Her grandmother started mild chorea of the four limbs at age 45 and died at 57. Her father was 36 years old without any signs so far despite his expanded CAG repeat number was 45.

During the course of disease, typical HD manifestations, like chorea, cognitive impairment, and psychological disorder gradually occurred in all four patients.

Neuroimaging

Cerebral atrophy was observed in all four patients on computerized tomography (CT) or magnetic resonance imaging (MRI). The abnormal signals were seen in bilateral basal ganglia of patients 1 and 4. In patient 4, the caudate and putamen nucleus were symmetrically atrophied while the slightly atrophic brainstem and cerebellum were also seen (Fig. 2).

Discussion

According to previous reports, we found those HD patients carrying CAG repeats beyond 60 had the following characteristics. First, they mainly had early onset. CAG repeats beyond 60 were responsible for approximately half of JHD and CAG repeats over 80 might cause childhood-onset HD (6, 18). Second, their manifestations were atypical, in which rigidity, bradykinesia, intellectual decline, behavior change, frequent falls, speech impairment, seizures, and ataxia were the initial or predominant (7, 14, 19–24). In addition, initial symptoms including excessive blinking, attention deficits, gait disorder, and global developmental delay were also observed (25–27). Third, paternal transmission was predominant pattern of inheritance (19). Fourth, the atrophies in cerebral cortex, striatum or cerebellum and increased density in T2-weighted signal in caudate and putamen nuclei in MRI were characteristic imaging features (12, 23, 28).

In this study, all four patients presented with atypical symptoms at early stage. Onset with epilepsy and intellectual decline were observed in two patients and non-choreiform movements occurred in the other two patients. Attention deficits, ataxia, dysphagia, gait disorder, bradykinesia and speech impairment were also observed. These features were in accordance with the characteristics summarized above. The neuroimaging features of our four patients were similar to those reported previously (12, 28). Unfortunately, the images of patients 1, 2 and 3 were missing.

Concerning about inheritance, three pedigrees showed paternal transmission except for pedigree 2 whose family history was obscure. In addition, rapid downhill course and familial clustering were found in pedigrees 1 and 3, whose family members were also severely affected. Conversely, this phenomenon was not observed in the pedigree 2 in which family history was absent. This interesting finding indicated clinical phenotypes could be different between the pedigrees with obvious family history and those with obscure family history. These preliminary data needs to be further confirmed in a larger cohort.

Patient 4 presented with walking instability in early stage of disease, which might be mistaken as SCA, since SCA was prevalent in Chinese population (29) and could also be juvenile onset (30). Although both JHD and juvenile-onset SCA patients might share the cerebellum signs, other features of HD were helpful to distinguish it from SCA, including saccade initiation delay, saccadic slowing, gaze impersistence and motor impersistence (7, 17, 29–32). Slow saccades were observed in patient 4, but other valuable signs like saccade initiation were not recorded.

In summary, among 119 Chinese HD patients, we identified four atypical patients carrying CAG repeats
Fig. 2. The brain magnetic resonance image (MRI) of patient 4 at the age of 14 years. The sagittal (a) sections reveal brainstem and cerebellum atrophy (arrow). (b–d) Atrophy and abnormal signals of the caudate and putamen nuclei (arrows) in T1 (b), T2 (c) and T2-FLAIR (d) weighted imaging.

beyond 60. The present report added information on the rare phenotype in Chinese HD patients carrying large CAG repeats and attempted to help the clinicians make a correct diagnosis in the early stage of disease.

Supporting Information

The following Supporting information is available for this article:

Fig. S1. The 1.5% agarose gel electrophoresis analysis of six Huntington’s disease (HD) patients from four families. M: DL2000 DNA molecular weight marker; 1: an identified HD patient (17/58); 2–5: patients 1–4 carrying CAG repeats beyond 60 (17/104, 18/82, 17/76, and 17/74); 6: the affected uncle (17/53) of patient 3; 7: the affected father of patient 4 (16/45).

Fig. S2. Chromatogram of patient 1 with CAG repeats of 17/104.

Fig. S3. Chromatogram of patient 2 with CAG repeats of 18/82.

Fig. S4. Chromatogram of patient 3 with CAG repeats of 17/76.

Fig. S5. Chromatogram of patient 4 with CAG repeats of 17/74.

Fig. S6. Chromatogram of H6 in pedigree 3 with CAG repeats of 17/53.

Fig. S7. Chromatogram of II1 in pedigree 4 with CAG repeats of 16/45.

Additional Supporting information may be found in the online version of this article.

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References


