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**Original article**

# Clinical and laboratory diagnosis of spinocerebellar ataxia type 3 in a large Chinese family

Sirui Yang[a], Weihong Xu[b], Shibo Li[b], Shicheng Liu[a], Honghua Lu[a], Xiaosheng Hao[a], Feiyong Jia[a], Guiling Xue[a]
*[a]Department of Pediatrics, the First Hospital of Jilin University, Changchun, Jilin, P. R. China 730021; [b]Department of Pediatrics, the University of Oklahoma Health Sciences Center, Oklahoma City, OK, 73104, USA*

***Background:*** Hereditary ataxia is a group of hereditary diseases that are characterized by chronic progressive uncoordinated gait and are frequently associated with cerebellar atrophy.
***Objectives:*** To investigate evidence-based diagnosis of hereditary ataxia by retrospective analysis of the diagnostic process in one Chinese family.
***Methods:*** Clinical records of 15 ataxia patients from one Chinese family with 46 family members were retrospectively reviewed and a tentative diagnosis was made based on clinical manifestations, signs and symptoms, mode of inheritance, and progression. Since hereditary ataxia is a group of heterogeneous diseases having various subtypes and overlapping symptoms, we adopted a stepwise evaluation to achieve a tentative diagnosis. To confirm the diagnosis, we performed polymerase chain reaction (PCR) specific for the suspected causative gene of spinocerebellar ataxia (SCA) subtype 3 (SCA3).
***Results:*** Through analysis of hereditary and clinical characteristics of family histories of the patients, we suspected that the family might suffer from SCA, especially, SCA3. The PCR assay for SCA3 showed that, five of the ten samples analyzed had a CAG trinucleotide expansion of the SCA3 gene, and four of the five members developed ataxia. The remaining one, a seven-year-old girl, showed no symptoms or signs except for uvula deviation. No clinical symptoms were found in five other members with negative PCR results. Thus, based on both clinical findings and laboratory results, we further confirmed that the family suffered from SCA3.
***Conclusion:*** Hereditary ataxias are disorders sharing overlapping symptoms. Comprehensive analysis of medical and family records together with genetic diagnosis improves diagnostic efficiency of hereditary ataxia and aides in family counseling.

***Keywords:*** Cerebellar ataxia, diagnosis, heredity, PCR

Hereditary ataxia is a group of diseases that are characterized by chronic progressive uncoordinated gait. They are frequently associated with cerebellar atrophy. Classification of hereditary ataxias depends mainly on the mode of inheritance, causative genes, and chromosomal loci. A number of hereditary ataxias have now been diagnosed, such as 16q22-linked spinocerebellar ataxia (SCA), ataxia with vitamin E deficiency, ataxia with oculomotor apraxia type 1, dentatorubral-pallidoluysian atrophy, Friedreich's ataxia; infantile onset SCA, and autosomal recessive spastic ataxia of Charlevoix-Saguenay. However, some hereditary ataxias can still not be identified with sophisticated molecular technology and a definite diagnosis is impossible in approximately 40-50% of cases [1-6].

Apart from different clinical manifestations and overlapping symptoms, various types of hereditary ataxias also differ in their causative genes or disease-associated chromosomal loci. SCAs are the most common form of hereditary ataxia and consist of 29 subtypes. These include subtypes 1-8 and 10-30. Subtype SCA9 has been reserved and no clinical or

***Correspondence to:*** Sirui Yang, MD, PhD, Department of Pediatrics, The First Hospital of Jilin University, 71 Xinmin Street, Changchun 730021, Jilin Province, P. R. China. E-mail: siruiyang@163.com

genetic information regarding this type has yet been published [5, 7, 8]. Except for a few early-onset cases, most SCAs occur after the age of 18 and disease severity may progress rapidly in some patients. In addition to common clinical features which include a progressive uncoordinated gait that is frequently accompanied by uncoordinated motion of hands or eyes as well as dysarthria and other pyramidal and extrapyramidal symptoms, differences are also found among various subtypes of SCAs [2, 3, 9]. For example, SCA1 often shows a significant active reflex (the age of onset ranges from less than 10 to over 60 years, with an average duration of 15 years). SCA2 may be associated with slow eye movement and occasionally with dementia (age of onset ranges from less than 10 to over 60 years). SCA3 may show reduced muscle strength and atrophy (age of onset ranges from 10 to 70 years, with an average duration of 10 years). SCA4 may present with sensory loss (age of onset ranges from 19 to 72 years, with an average duration of several decades). SCA5 occurs at younger ages and progresses very slowly (age of onset ranges from < 10 to > 60 years, with an average duration of 25 years). SCA7 may be accompanied by visual loss (age of onset ranges from less than 10 to over 60 years, with an average duration of 20 years). SCA8 may present with active reflexes and decreased sensation (age of onset ranges from 18 to 65 years, normal lifespan). SCA10 may be associated with occasional seizures (age of onset at mid life, with an average duration of nine years). SCA11 usually shows mild symptoms and patients remain ambulatory (age of onset at mid life with a normal lifespan). SCA12 shows slowly progressive ataxia, action tremors in the 30s, hyper-reflexia, occasional subtle Parkinsonism and cognitive/psychiatric disorders including dementia (age of onset ranges from eight to 55 years) [2, 3]. SCA13 is associated with slight mental retardation and short stature (childhood onset) [2, 3, 9].

We investigated a large five-generation family with members suffering from SCA3. To make an accurate diagnosis, we performed a stepwise evaluation program using different factors, such as hereditary patterns, clinical features, age of onset, and duration of disease. The results of our study showed the variety of symptoms and signs of SCA3 in this large family and the presence of SCA3 in this family were finally confirmed by PCR.

## Materials and methods

We recruited five generations of one family for this study. **Figure 1** shows a pedigree of the family, and **Table 1** provides a summary of the clinical phenotypes observed in some of the family members. The present study was conducted in accordance with international guidelines and was approved by the Ethics Committee of the First Hospital of Jilin University.

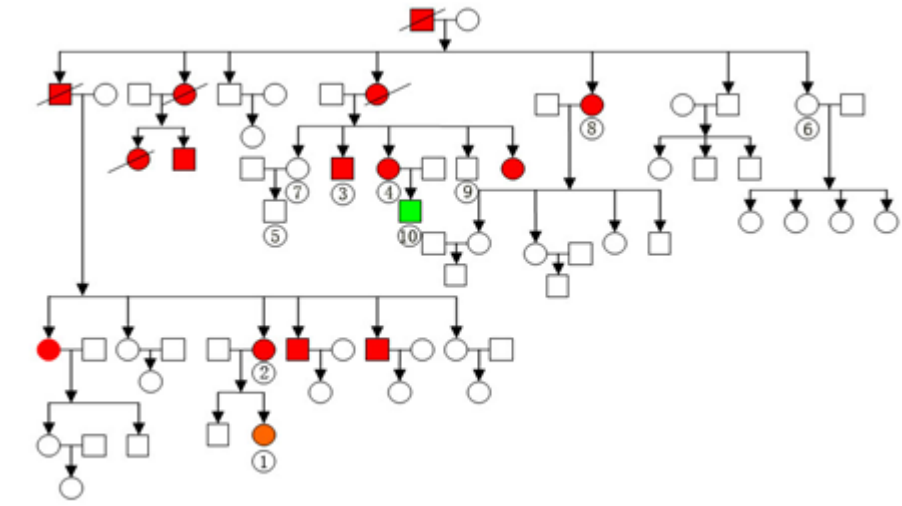


**Fig. 1** A pedigree chart showing the SCA3-affected family members and the number of patients who received genetic diagnosis. Numbers one to ten represent family members who provided a blood sample, and this numbering system was the same as that used in the PCR assay. Red-filled symbols represent family members with ataxia. The green-filled symbol represents a family member with progressive muscular atrophy. The brown symbol (#1) represents the seven-year-old girl without symptoms or signs, except for uvula deviation. No filled symbols represent family members not showing symptoms or signs. The square symbols represent males and the circle symbols females. Slash represents family member died of SCA3.

**Table 1.** Clinical phenotypes and PCR results of the 10 affected family members. Patient No.1 had no symptoms except uvula deviation and was positive for SCA3 in the PCR test. Patient numbered 2, 3, 4, and 8 showed symptoms and were positive for SCA3 in the PCR test. No abnormal clinical manifestations or signs appeared in patients numbered 5, 6, 7, 9, and 10, and their PCR results were negative for SCA3. F represents female; M represented male. An empty blank represented negative sign. ↑ represented enhanced or increased. Classification of muscle strength was based on the following scale: 0: complete paralysis, no movement; I: slight muscle contraction; II: horizontal movement of limb on the bed. III: limbs can lift from the bed; IV: limb can move to act against external resistance; V: normal muscle strength, free movement.

| Patient No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Age (year) | 7 | 38 | 39 | 29 | 17 | 58 | 42 | 50 | 27 | 8 |
| Gender | F | F | M | F | M | F | F | F | M | M |
| Age of onset | | 34 | 25 | 23 | | | | 39 | | |
| Unsteady gait | | + | + | + | | | | + | | |
| Cough after drinking | | | + | + | | | | | | |
| Vertigo | | | | + | | | | | | |
| Diplopia | | | | | | | | | | |
| Nystagmus | | | + | + | | | | | | |
| Dysarthria | | | | + | | | | | | |
| Tinnitus | | | | + | | | | | | |
| Alalia | | | | + | | | | | | |
| Facial paralysis | | | + | + | | | | + | | |
| Tongue deviation | | | + | + | | | | | | |
| Uvula deviation | + | + | + | + | | | | | | |
| Finger-nose test | | | + | + | | | | | | |
| Romberg's sign | | | + | + | | | | | | |
| Superficial sensory disturbance | | | + | + | | | | + | | |
| Knee jerk reflex | | | + | ‘! | | | | | | |
| Ankle clonus | | | | + | | | | | | |
| Muscle strength | V | V | III | V | | | | IV | | |
| Muscle tone | | | ↑ | ↑ | | | | | | |
| Muscle atrophy | | | + | + | | | | | | |
| PCR | + | + | + | + | - | - | - | + | - | - |

Of the 46 family members studied, 15 (six males, nine females, age range of onset seven to 53 years, average 33 years) developed progressive ataxia. Of the five patients who died, the average natural duration was 11.7 years (range six to seventeen years) from onset to death. Patient No.1 was a seven-year-old girl who showed no symptoms or signs except uvula deviation. Patient No.10 was an eight-year-old boy suffering from severe Duchenne progressive muscular dystrophy, which seemed to be unrelated to the progressive ataxia in this family. Magnetic resonance imaging (MRI) showed cerebellar atrophy in patient No. 4 **(Fig. 2).**

***Procedures for progressive evaluation***

Diagnosis was sequentially narrowed down using the following progressive approach: **(1) Mode of inheritance**: The disease in the family was diagnosed to be an autosomal dominant inherited condition, as shown in **Figure 1**. Diseases other than autosomal dominant inherited diseases could be safely excluded. (**2) Clinical manifestations**: As shown in **Table 1**, general clinical manifestations in this family included progressive ataxia, pyramidal signs, and extrapyramidal syndromes, such as hypertonia. If these were present, SCA was tentatively diagnosed [9, 10]. (**3) Age of onset and duration**: The age of onset ranged between seven to 53 years, with an average of 33 years. The duration of the disease ranged from six to seventeen years, with an average of 11.7 years. These characteristics indicate that the disease was most likely SCA1, SCA2, or SCA3 [2, 9, 11]. **(4)**

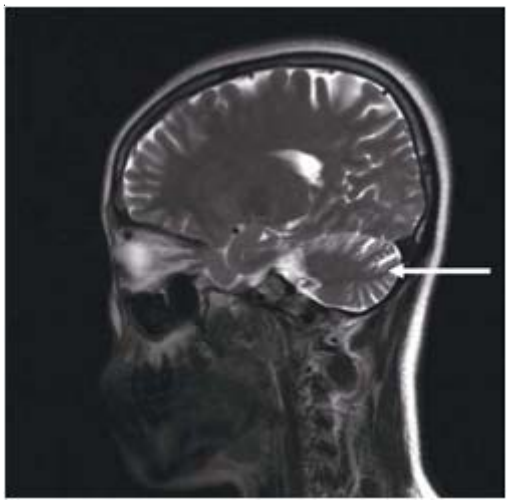

**Fig. 2** A representative magnetic resonance imaging (MRI) image of patient No. 4 showing obviously widened and deepened cerebellar sulci, suggesting the presence of cerebellar atrophy, as indicated by the white arrow. Major clinical manifestations of the 29-year-old female patient are presented in **Table 1.**

**Frequency of onset**: based on the fact that the frequency of SCA3 onset (referring to the percentage of SCA3 patients relative to the total patients with SCAs) was the highest among the subtypes (SCA1 5.8%, SCA2 6.7%, SCA3 49.2% in China [12]), the disease was more likely to be diagnosed as SCA3. (**5) Re-evaluation of clinical manifestations**: Reduced muscle strength and muscle atrophy occurred, both of which are characteristic of SCA3 [2, 9, 10].

### *Genetic diagnosis*

After conducting the evaluation process described above, we tested whether the disease was SCA3 using previously described procedures [12]. In brief, blood samples were collected from 10 patients (No. 1-10) as shown in **Fig. 1** and **Fig. 3** and total DNA was extracted and used as a template for polymerase chain reaction (PCR). The primer pair sequences of SCA3 were as follows: forward: 52 CCAGTGACTACTT TGATTCG32; reverse: 52 TGGCCTTTCACATGG AT32 . The PCR amplification was performed in 25 µl of the PCR reaction system containing 2.5 µl of 10 x buffer; 0.5 µl of 25 mM $MgCl_2$; 0.5 µl of 10 mM dATP, dCTP, and dTTP; 0.25 µl of 10 mM dGTP; 0.25 µl of 10 mM 7-deaza dGTP; 1 µmol/L primers; 2.5 U Exo(-) Pfu enzyme (Stratagene, La Jolla, CA, USA); 100 ng DNA template, and double distilled water. The PCR reaction system was first pre-denatured at 94°C, followed by 33 cycles at 94 °C for one min, 58 °C for one min, and 72°C for 30 sec, and a final extension step at 72 °C for 10 min. The PCR amplification was performed in a TC200 PCR thermocycler (MJ Research, Watertown, MA USA), and amplified PCR products were separated on a 2% agarose gel. The image was recorded using a Polaroid GelCam (Polaroid, Cambridge, MA, USA) under UV light provided by a UV transilluminator (UVP, Upland, CA, USA).

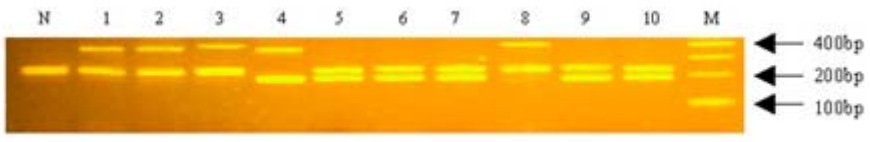


**Fig. 3** This gel illustrates the PCR amplification of the SCA3 gene in 10 patients from the SCA3 family. N represents a negative control sample from healthy person outside of the family. M represents marker. In the patient numbered 1, 2, 3, 4, and 8, the PCR product of one allele was about 200 bp, whereas that of another allele was about 400 bp. In the patient samples numbered 5, 6, 7, 9, and 10, the PCR product of both alleles was around 200 bp.

## Results

We found two PCR product bands in the patient numbered 1, 2, 3, 4, and 8 **(Fig. 3)**. One band was about 200 bp in length, which represented the fragment of the normal allele without the expansion of CAG trinucleotide repeats; the other band was about 400 bp, representing the expansion of the CAG trinucleotide repeat in the allele. This result indicated that these samples are positive for SCA3 in the PCR assay, though patient No. 1 was a seven-year-old girl who had no clinical manifestations of SCA3 upon physical examination except for lateral deviation of the uvula. Whereas in the patient numbered 5, 6, 7, 9, and 10, the PCR products for both alleles were around 200 bp. This finding suggests that there is no expansion of the trinucleotide CAG in the two alleles from these family members, which is consistent with their normal clinical phenotypes for SCA3, though patient No. 10 presented with severe Duchenne progressive muscular dystrophy.

## Discussion

Despite marked progress in the study of hereditary ataxias, accurate diagnosis remains challenging. Clinical and laboratory data need to be utilized more efficiently and systematically. Although overlapping symptoms occur in various subtypes of hereditary ataxias, the clinical manifestations of each subtype are different due to differences in mutational sites. In recent years, the clinical features of some forms of ataxias have been well documented [2, 9, 11] and provide a basis for evidence-based diagnosis.

Determination of the mode of inheritance is a relatively simple process. It is important and needs to be performed first. As suggested in **Fig. 1**, the family suffered from an autosomal dominant inherited disease. Moreover, we found that the general clinical features, including progressive ataxia, pyramidal signs, and extrapyramidal syndromes (e.g., hypertonia), are characteristic of SCAs [9]. To date, although 29 subtypes of SCAs have been classified [2, 9], it still remains difficult to make a differential diagnosis of these subtypes. Based on the characterizations of the age of the onset and the duration of the disease, we concluded that the 15 affected patients in our study most likely had SCA1, SCA2, or SCA3. Moreover, SCA3 was the most likely diagnosis due to its highest frequency of onset among all subtypes of SCAs in China [12]. Based on the clinical features of each subtype, as reported previously [2, 9-11], and the results presented in **Table 1**, we re-evaluated the clinical features of these patients in more details. We found that the clinical manifestations present in the members of this Chinese family, including progressive ataxia, unsteady gait, nystagmus, dysarthria, muscular atrophy, and reduced muscle strength, were in accordance with the clinical features of SCA3 [2]. Our diagnosis was also further confirmed by PCR tests.

SCA3 (Machado-Joseph disease, MJD) is a progressive autosomal dominant hereditary neurodegenerative disease. It was first reported by Nakano et al. [13] and Rosenberg et al. [14]. Its causative gene was mapped to 14q32.1 by Kawaguchi et al. [8]. The onset of SCA3 is linked to an unstable expansion of the CAG trinucleotide, which has 13-36 repeats in the normal population but expands to 68–79 repeats in affected patients; the corresponding PCR products in affected patients are 186–230 bp and 384–405 bp. Our PCR results were consistent with these previous findings. Of the five patients confirmed by genetic diagnosis, patient No. 1 showed no symptoms, possibly due to her young age. However, lateral deviation of the uvula was detected in this patient, suggesting that the deviation might be an early manifestation of this disease. Currently, no specific treatment regimen is available for this disease, although Artane or L-dopa has been used for dystonia or other extrapyramidal symptoms [9].

## Conclusion

We found that the diagnostic process using a systematic approach together with historical and physical findings is useful for evaluating this familial disease. Furthermore, it was confirmed by molecular genetic technology.

The authors have no conflict of interest to declare.

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