The dynamic mutation investigation and whole exome sequencing in a cohort of Chinese autosomal dominant cerebellar ataxia patients

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Abstract

Background Spinocerebellar ataxias (SCAs) are the autosomal dominant cerebellar ataxia (ADCA) with great clinical and genetic heterogeneity. Genetic testing will contribute to the final diagnosis. Methods A total of 204 Chinese ADCA patients were recruited and 190 had genetic testing. Dynamic mutations of SCA1, 2, 3, 6, 7, 8, 10, 12, 17 and dentatorubral-pallidoluysian atrophy (DRPLA) were screened firstly. For the patients with negative results, the dynamic mutations of HTT of Huntington Disease (HD), SCA31, 36 and even the whole exome sequencing (WES) were further performed. We investigated the genetic results and clinical characteristics retrospectively.

Results Among these 190 index cases, 177(93.16%) were identified SCA dynamic mutations. SCA3 was the commonest, accounting for 70.06%, followed by SCA1 (9.6%), 2 (9.05%), 12 (3.39%), 6 (2.26%), DRPLA (2.26%), 7(1.13%), 8 (1.13%) and 17(0.56%). One patient carried a compound dynamic mutation of SCA6 and SCA17 (SCA6/17). No SCA10 or SCA36 was found. Among the remaining 13 patients, three were diagnosed with HD (1.58%) and one with Episodic Ataxia 2 (EA2). WES did reveal several variants with uncertain significance (VUS) in the remaining nine patients, but failed to detect causative mutations.

Conclusion We illustrated the approach and challenge of genetic testing in Chinese ADCA patients. Dynamic mutations of SCAs should be screened firstly. When the results were negative, dynamic mutation of HTT would better be screened consequently. In early-onset ADCA patients, WES might be effective to identify causative mutations, but in adult-onset cases, WES might be less effective.

1. Introduction

Spinocerebellar ataxias (SCAs) are the autosomal dominant cerebellar ataxia (ADCA) with
progressive cerebellar ataxia combined with other manifestations such as peripheral neuropathy, ophthalmoplegia, pigmentary retinopathy, pyramidal signs, extrapyramidal symptoms and cognitive impairment[^1]. Up to date, more than 40 SCA subtypes including dentatorubral-pallidoluysian atrophy (DRPLA) and DNMT1[^2] have been reported. Since the diverse clinical heterogeneity of SCAs, it is difficult to distinguish different subtypes on the basis of clinical features. Therefore, genetic testing will contribute to the final diagnosis.

SCAs can be caused by both dynamic mutations and non-repeat mutations[^3]. SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36, 37 and DRPLA were caused by dynamic mutations[^2]. Though the prevalence of each subtype is variable in different ethnic and regions, the SCA1-3, 6 and 7 are much more common worldwide[^4–7]. According to the previous reports in Chinese ADCA patients, SCA3 accounted for 12.5%-72.5%^[^8, 9], followed by SCA2 (5.88–6.7%), SCA1 (4.7–5.8%)[^9, 10], SCA6 (1.6–3.3%), SCA7 (0.8–4.8%)[^11, 12] and SC8 (0.59%)[^13]. DRPLA only accounted for 0.36% in a large-scale genetic screening[^14]. SCA36 accounted for 0.6%^[^15]. SCA10, 12, 17 and 31[^16] were mainly reported as cases. No SCA37 was identified[^17]. In addition to the dynamic mutations of SCAs, the dynamic mutation of HTT of Huntington Disease (HD) can also lead to ataxia[^18], which should be paid attention to.

Non-repeat mutations, especially missense mutations are another important cause of ADCA. Whole exome sequencing (WES) has been adopted as a cost-effective and high-yield way to discover the non-repeat mutations related to ataxia, which might help to confirm the diagnoses of ADCA patients with negative SCA or HD dynamic mutations[^19, 20].
Here we reported the genetic and clinical characteristics of 190 Chinese ADCA patients. We illustrated the approach of genetic testing and discussed the significance and challenge of performing WES, which would help clinicians prioritize genetic testing.

2. Materials And Methods

2.1 Subject and clinical materials

ADAC patients visiting the movement disorder clinic or neurogenetics clinic in Huashan Hospital were enrolled continuously from August 2014 to March 2019. All the participants signed informed consent before participation. The study was approved by the Human Studies Institutional Review Board, Huashan Hospital, Fudan University.

2.2 Genetic analyses

Genomic DNA was extracted from the peripheral blood leucocytes of the participants (Qiagen, Germany). Triplet repeat primers polymerase chain reaction (TP-PCR) and Capillary Electrophoresis Techniques were used to detect dynamic mutations, including SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36 and DRPLA[21]. The triplet nucleotide repeat expansion of HTT was carried out by PCR and Sanger sequencing[18].

The WES was carried out by Sure Select Human All Exon Kit V5 (Agilent, Santa Clara, CA) and high throughput sequencing by Illumina novasEq. The quality control and the variant screening were as previously reported[22].

The flowchart of genetic testing was shown in Fig. 1.

3. Results

3.1 Demographic characteristics of the patients

A total of 204 index patients were recruited and 190 performed genetic testing. Among them, 104 were male and 86 were female. The average onset age was 35.83 ± 9.32.
3.2 Results of the genetic testing

The mean depth of the WES was 107.488X. The average percentage of the target region with mean depth > 20X was 96.35%.

A total of 177 participants (93.16%) were found carrying SCAs dynamic mutations. The demography and frequencies of SCA subtypes were shown in Table 1. SCA3 was the commonest, followed by SCA1, 2, 12, 6, DRPLA, 7, 8, 17. One patient carried a compound dynamic mutation of SCA6 and SCA17 (SCA6/17). Among the remaining 13 patients, three were diagnosed with HD, one with Episodic Ataxia 2 (EA2), six carrying variants with uncertain significance (VUS), three with negative results. No SCA10 or 36 was found (Fig. 2).

Table 1
Demography of the patients carrying SCA dynamic mutations and their frequencies of related SCA subtypes

| SCA subtype | Number of patients | Frequency of SCA subtypes | Expanded multi-nucleotide repeat number, mean ± SD (range) | Gender (M/F) | Age at onset, mean ± SD (range) |
|-------------|--------------------|---------------------------|----------------------------------------------------------|-------------|---------------------------------|
| SCA1*       | 17                 | 9.6%                      | 49.3 ± 8.04 (30-65)                                       | 12/5        | 34.6 ± 9.85 (17-50)             |
| SCA2        | 16                 | 9.05%                     | 41.8 ± 2.93 (20-49)                                       | 6/10        | 32.6 ± 8.72 (20-51)             |
| SCA3        | 124                | 70.06%                    | 68.6 ± 6.45 (37-82)                                       | 72/52       | 37.9 ± 10.11 (14-63)            |
| SCA6        | 4                  | 2.26%                     | 24.5 ± 2.38 (21-26)                                       | 2/2         | 47.5 ± 12.07 (31-60)            |
| SCA7        | 2                  | 1.13%                     | 43.5 ± 0.71 (43-44)                                       | 0/2         | 30.0 ± 12.73 (21-39)            |
| SCA8        | 2                  | 1.13%                     | 94.5 ± 6.36 (90-99)                                       | 1/1         | 46.5 ± 19.09 (33-60)            |
| SCA12       | 6                  | 3.39%                     | 45.3 ± 2.58 (43-48)                                       | 5/1         | 52.3 ± 9.91 (34-61)             |
| SCA17       | 1                  | 0.56%                     | 48                                                        | 0/1         | 46                              |
| SCA6/17     | 1                  | 0.56%                     | 35, 41                                                   | 0/1         | 28                              |
| DRPLA       | 4                  | 2.26%                     | 61.3 ± 6.34 (54-63)                                       | 3/1         | 41.5 ± 18.23 (29-68)            |
| Total       | 177                | 100%                      | /                                                         | 101/76      | 38.0 ± 10.83 (14-68)            |

SCA: spinocerebellar ataxia; SD: standard deviation.
*The CAG repeat numbers of five SCA1 patients were less than 45 but the CAG triplet repeats were proven without CAT repeat interruption by Sanger sequencing and were ascribed pathogenic.

3.3 Clinical characteristics of patients with certain diagnoses

3.3.1 Patients presenting with positional tremor
Positional tremor was the most noticeable symptom of SCA12, which could serve as a clue to confirm SCA12. In our cohort, 80% of SCA12 patients had positional tremor in the head or hands. 19% of SCA1 and 33% of SCA2 patients also complained about positional tremor.

3.3.2 Patients presenting with saccadic movement disorders

Saccadic movement disorders include saccadic starting slowly, sudden setback in the process and dysmetria. 73.3% of SCA2, 50% of SCA1 and 33.7% of SCA3 patients developed saccadic movement disorders.

3.3.3 Patients presenting with visual impairment

Both SCA7 patients presented with severe visual impairment. One patient could hardly read and the other was diagnosed with retinal pigmentosa by optical coherence tomography (OCT). 11.8% of SCA1 and 6.9% of SCA3 patients showed blurred vision.

3.3.4 Patients presenting with psychiatric symptoms

Three patients (11.1%) in four DRPLA pedigrees showed irritability. SCA17 could not cooperate well with the physical examination because of emotional swings and temper tantrum.

3.3.5 Patients presenting with Parkinsonism

SCA3 has been classified into several subgroups previously\(^{[23]}\). Our patients fell into three subgroups of levodopa responsive Parkinsonism, ataxia combined with pyramidal signs and peripheral neuropathy. Five SCA3 patients showed Parkinsonism, accounting for 5%, manifesting as bradykinesia, rigidity, and postural instability. One male SCA8 patient developed a typical Progressive Supranuclear Palsy (PSP) phenotype with bradykinesia, rigidity, festination and freezing gait.

3.3.6 Patients presenting with epilepsy

Nine patients (33.3%) in our four DRPLA pedigrees (Fig. 3) presented with epilepsy. The
detailed clinical manifestations were concluded in Table 2.

**Table 2**
The clinical features of the probands with DRPLA and their family members

| Pedigree | Patient number | Onset age (years old) | Current age (years old) | Situation at investigation |
|----------|----------------|-----------------------|-------------------------|---------------------------|
| Family A | I:2            | 75                    |                         | Unstable walking, died at the age 87 |
|           | II:1           | 50                    | 69                      | Unstable walking, died at the age 75  |
|           | II:4 (the proband) | 68                    |                         | Unstable walking          |
| Family B | I:1            | NA                    | 30                      | Unstable walking, died at the age 59 |
|           | II:1           | 30                    | 45                      | Unstable walking, died at the age 39  |
|           | II:2 (the proband) | 19                    |                         | Epilepsy, unstable walking   |
|           | III:2          | 22                    |                         | Epilepsy, unstable walking   |
| Family C | II:1           | 50                    | 44                      | Unstable walking, died at the age 69  |
|           | III:1 (the proband) | 39                    | 33                      | Unstable walking, involuntary movement,  |
|           | II:2           | < 15                  | 30                      | irritability               |
|           | III:5          | 30                    | 25                      | Epilepsy, unstable walking, dysphonia,  |
|           | III:7          | 30                    | 23                      | bed-ridden, died at age 22          |
|           | III:8          | 25                    | Youth                   | Epilepsy, unstable walking   |
|           | III:9          | 21                    | Youth                   | Epilepsy, unstable walking, cognitive impairment from youth |
|           | IV:2           | 21                    | Youth                   | Cognitive impairment       |
|           | IV:3           | 26                    | Youth                   | Epilepsy                   |
|           | IV:4           | 24                    | Youth                   | Cognitive impairment       |
|           |                | 26                    |                         | Epilepsy                   |
|           |                | 24                    |                         | Cognitive impairment       |
|           |                | 23                    |                         | irritability               |
| Family D | II:1           | Youth                 | 77                      | Unstable walking           |
|           | II:2           | Youth                 | 72                      | Unstable walking           |
|           | II:3           | Youth                 | 72                      | Unstable walking           |
|           | II:4           | Youth                 | 72                      | Epilepsy, slurred speech    |
|           | III:1          | Youth                 | 46                      | Unstable walking           |
|           | III:2          | Youth                 | 43                      | Unstable walking           |
|           | III:3          | Youth                 | 41                      | Unstable walking           |
|           | III:4 (the proband) | Youth                 | 39                      | Epilepsy, unstable walking, chorea,  |
|           | IV:1           | 10                    | -                       | slurred speech             |
|           | IV:2           | 5                     | -                       | Cognitive impairment       |
|           |                |                        |                         | Cognitive impairment       |
|           |                |                        |                         | Cognitive impairment       |
|           |                |                        |                         | Cognitive impairment       |

DRPLA: dentatorubral-pallidoluysian atrophy; NA: not available, -: the patient has died

3.3.7 Patients presenting with cognitive impairment

Eight patients (29.6%) in four DRPLA pedigrees showed cognitive impairment (Table 2).
The SCA17 patient showed severe cognitive decline with Mini-Mental State Examination (MMSE) score 4/30. The PSP-phenotype SCA8 patient showed moderate cognitive impairment with MMSE score 14/30. The MMSE score of the SCA6/17 patient was 23/30 (14 years of education).

### 3.3.8 Clinical characteristics of HD patients

Three HD patients were detected in our study. The average onset age was 45.67 ± 7.37. Their repeat numbers of HTT were 46, 46 and 43 respectively. All of them presented with unstable walking and blurred speech at early stage. One patient developed chorea and cognitive decline later and the other two were still dominated by cerebellar ataxia. These atypical clinical symptoms would often lead to the initial misdiagnosis.

### 3.4 The results of WES

One patient was diagnosed with EA2 after performing WES. A heterozygous frameshift mutation of c.2764-2765insc (p.Arg922ProfsTer145, NM_023035) in CACNA1A was revealed, which has not been reported and according to the American College of Medical Genetics and Genomics (ACMG) guideline, it was rated as likely pathogenic (PM2 + PM4 + PP3 + PP4). But in the remaining nine patients, except for some VUS, no causative mutations were found. So the detection rate of WES was 10%. We concluded the clinical manifestations and predicted the pathogenicity of these VUS in Table 3.

Table 3: Results of the whole exome sequencing and clinical features of the probands

| Patient No. | Gender | Onset age | Current age | Clinical symptoms and signs | Gene | Variant & Transcript | Mutation & Taster prediction | ACMG variant rating | Related phenotypes |
|-------------|--------|-----------|-------------|----------------------------|------|----------------------|--------------------------|-------------------|-------------------|
| I F         | 60     | 61        | Unstable walking, slurred speech | SLC1A3 (NM_004172) | c.397 A > G | p.Ile133Val | Het | Disease causing Benign 0.46 | Tolerated 0.004 | NA | NA | VUS (PM2) | EA6[1] |
| II F        | 44     | 48        | Unstable walking, slurred speech | OPAL1 | c.598_603delAAG & c.598_602delAAA | Het | Polymorphism | NA | NA | NA | NA | VUS (PM2) | OAPS[1] |
|   |   |   | Unstable walking, slurred speech, bucking, blurred vision, horizontal nystagmus, tremor in hands, limbs ataxia | TTBK2 (NM_015560.2) c.1359A>C & p.Lys453Asn | Het | Diseasem | Benign | 0.00 | Deleterious | NA | NA | VUS (PM2) | SCA11[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| III | M | 45 | 46 | Unstable walking, slurred speech, limbs ataxia, hyperreflexia | TTBK2 (NM_01573500.3) c.7G>C & p.Gly3Arg | Het | Diseasem | Possibly damaging | 0.00 | Deleterious | NA | 0.0162% | NA | VUS (PM2+PP3) | SCA11 |
| Group | Gender | Age | Features |
|-------|--------|-----|----------|
| VII   | M      | 50  | Difficulty in walking, spastic gait, hyperreflexia, hypermyotonia, bilateral positive pathologic signs, normal MRI and EMG results |
| VIII  | F      | 46  | Unstable walking, slurred speech, bucking, limb ataxia, hyperreflexia, serious decline in vision, ptosis, limited eye movements, macular |
|   |   |   |   | Unstable walking, spastic ataxia gait, hyperreflexia, hypertonia, negative pathological signs, slurred speech, bucking, tongue amyotrophy and fibrillation, EMG revealed chronic neurogenic myoelectric change, involving upper and lower limb muscles, suggesting damage to motor neurons or axons |

Abbreviations: ACMG: The American College of Medical Genetics and Genomics; BP: Benign
criterion is weighted as supporting; CANPMR: Non-progressive cerebellar ataxia with mental retardation (OMIM:614756); EA6: Episodic ataxia type 6 (OMIM:612656); EMG: Electromyography; F: Female; Het: Heterozygous; Hom: homozygous; M: Male; MRI: Magnetic resonance imaging; NA: not available; No.: number; OAPS: Optic atrophy plus syndrome (OMIM:125250); OMIM: Online Mendelian Inheritance in Man; PM: Pathogenic criterion is weighted as moderate; PP: Pathogenic criterion is weighted as supporting; SCA11: Spinocerebellar ataxia-11 (OMIM:604432); VUS: Variants of uncertain significance;

Reference
[1]. Jen JC, Wan J, Palos TP, et al. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. Neurology. 2005, 65(4):529-34.
[2]. Treft RL, Sanborn GE, Carey J, et al. Dominant optic atrophy, deafness, ptosis, ophthalmoplegia, dystaxia, and myopathy. A new syndrome. Ophthalmology. 1984,91(8):908-15.
[3]. Houlden H, Johnson J, Gardner-Thorpe C, et al. Mutations in TTBK2, encoding a kinase implicated in tau phosphorylation, segregate with spinocerebellar ataxia type 11. Nat Genet. 2007,39(12):1434-6.
[4]. Thevenon J, Lopez E, Keren B, et al. Intragenic CAMTA1 rearrangements cause non-progressive congenital ataxia with or without intellectual disability. J Med Genet. 2012,49(6):400-8.

4. Discussion
As a single-center study, we recruited cerebellar ataxia patients with a dominant inheritance consecutively. To investigate the relationship between genotypes and clinical phenotypes, we developed a genetic testing program. Dynamic mutations of SCA1, 2, 3, 6, 7, 8, 10, 12, 17 and DRPLA were screened firstly. Then the dynamic mutations of HD, SCA31, 36 and even the WES were further performed.
In this research, we found that SCAs were the overwhelming majority. Perhaps the limitation of dominant inheritance increased the frequency of SCAs. On the other hand, the incidence of HD in our cohort was 1.58%, no less than some SCA subtypes, suggesting that HD should be considered when patients manifested as cerebellar ataxia. When dynamic mutations of SCAs were negative, the dynamic mutation of HTT would better be detected subsequently.

As for clinical manifestations, some typical signs could help discriminate different subtypes[2], such as saccadic movement disorders in SCA2, vision impairment in SCA7, positional tremor in SCA12, chorea, epilepsy and dementia in DRPLA, psychiatric symptoms and dementia in SCA17. Parkinsonism could occur in SCA3, but in SCA8 it has been reported as a rare phenotype[26, 27], suggesting SCA8 should not be ignored when dealing with parkinsonism. SCA31 and SCA36 were really rare in Chinese population, no SCA37 reported. Therefore, we detected SCA31 and SCA36 in the remaining ten patients. SCA31 testing failed for some reasons. No SCA36 was detected. SCA31 usually had ataxia, dysarthria, decreased muscle tone, sometimes showing sensorineural hearing loss[28]. SCA37 was characterized by ataxia with distinctive abnormal ocular movements, including dysmetric vertical and horizontal saccades and pursuit, which were apparent in the early stage, even as a pre-symptomatic feature[29]. By reviewing all the clinical characteristics of the remaining patients, no patient was consisted with the phenotypes of SCA31 and SCA37.

WES has been recognized as effective to identify the pathogenic missense mutations in cerebellar ataxia patients, with the overall detection rate 18%-41%[20, 30]. And in early-onset (≤ 20 years old) patients, the detection rate was much higher with 39%-46%[31, 32], especially in familial cases, the rate was up to 69%-75%[30, 32]. These data suggested WES
was effective in early-onset patients. While in adult-onset cases, the detection rate was much lower, only accounting for 8.3%\textsuperscript{[30]} and 15%\textsuperscript{[19]}. In 183 Chinese cases excluded for dynamic mutations, the diagnostic rate was only 9.83%, suggesting the frequencies of non-polyglutamine SCA cases might be originally low in China\textsuperscript{[13]}. In our study, the detection rate was 10% and the remaining nine patients were all adult-onset patients (> 40 years old), which might not be the preferred population for WES. These remaining patients might be caused by novel genetic abnormalities, including new dynamic mutations or non-repeat mutations, or the mitochondrial mutations which had not been screened. Therefore, in cerebellar ataxia patients, especially in adult-onset ADCA patients, WES might not play such a huge role in the final diagnosis. There still be a high possibility to discover novel genetic mutations, which would broaden the spectrums of genotypes and phenotypes related to ataxia.

5. Conclusion

In this study, we illustrate the approach and challenge of genetic testing in Chinese ADCA patients. Dynamic mutations of SCAs are the main cause and should be screened firstly. If the results were negative, dynamic mutation of HD would better be performed consequently. For further genetic testing, early-onset patients or patients with symptoms directed to certain non-repeat mutations may benefit from WES. But in adult-onset patients, WES might be less effective and novel cerebellar ataxia related genes are still to be expected, which need our further research.

Abbreviations

ACMG: The American College of Medical Genetics and Genomics; BP:Benign criterion is weighted as supporting; CANPMR:Non-progressive cerebellar ataxia with mental retardation (OMIM:614756); EA6:Episodic ataxia type 6 (OMIM:612656);
EMG: Electromyography; F: Female; Het: Heterozygous; Hom: homozygous; M: Male; MRI: Magnetic resonance imaging; NA: not available; No.: number; OAPS: Optic atrophy plus syndrome (OMIM: 125250); OMIM: Online Mendelian Inheritance in Man; PM: Pathogenic criterion is weighted as moderate; PP: Pathogenic criterion is weighted as supporting; SCA11: Spinocerebellar ataxia-11 (OMIM: 604432); VUS: Variants of uncertain significance;

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Huashan Hospital. Written informed consents for participation were obtained from both patients and their relatives.

Consent for publication

Written informed consents for publication were obtained from both patients and their relatives.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Competing Interests

The authors declare that they have no competing interests.

Authors’ contributions

Fang Peng performed the statistical analysis and drafted the manuscript. Yue Zhang participated in the design of the study and performed molecular genetic studies. Xin-Yue Zhou performed molecular genetic studies. Shuai-Qi Huang participated in the design of the study. Chen Chen participated in the design of the study. Zheng-Tong Ding collected the clinical data. Jian Wang collected the clinical data. Yi-Min Sun carried out the molecular genetic studies and drafted the manuscript. Jian-Jun Wu
conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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References

[1]. Schöls L, Amoiridis G, Büttner T, et al. Autosomal Dominant Cerebellar Ataxia: Phenotypic Differences in Genetically Defined Subtypes? Ann Neurol. 1997, 42(6):924-32.

[2]. Sullivan R, Yau WY, O'Connor E, et al. Spinocerebellar ataxia: an update. J Neurol. 2019, 266(2):533-44.

[3]. Klockgether T, Mariotti C, Paulson HL. Spinocerebellar ataxia. Nat Rev Dis Primers. 2019, 5(1):24.

[4]. Jardim LB, Silveira I, Pereira ML, et al. A survey of spinocerebellar ataxia in South Brazil-66 new cases with Machado-Joseph disease, SCA7, SCA8, or unidentified disease-causing mutations. J Neurol. 2001, 248 (10):870-6.

[5]. Velázquez Pérez L, Cruz GS, Santos Falcón N, et al. Molecular epidemiology of spinocerebellar ataxias in Cuba: insights into SCA2 founder effect in Holguin. Neurosci Lett. 2009, 454(2):157-60.

[6]. Teive HA. Spinocerebellar degenerations in Japan. New insights from an epidemiological study. Neuroepidemiology. 2009, 32(3):184-5.

[7]. Bryer A, Krause A, Bill P, et al. The hereditary adult-onset ataxias in South Africa. J Neurol Sci. 2003, 216(1):47-54.

[8]. Jiang M, Jin CL, Lin CK, et al. Analysis and application of SCA1 and SCA3/MJD gene CAG repeats in Han population in Northeastern China. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2004, 21(1):83-5.

[9]. Gan SR, Shi SS, Wu JJ, et al. High frequency of Machado-Joseph disease identified in southeastern Chinese kindreds with spinocerebellar ataxia. BMC Med Genet. 2010, 11:47.
[10]. Jiang H, Tang BS, Xu B, et al. Frequency analysis of autosomal dominant spinocerebellar ataxias in mainland Chinese patients and clinical and molecular characterization of spinocerebellar ataxia type 6. Chin Med J. 2005, 118(10):837-43.

[11]. Wang J, Xu Q, Lei L, et al. Studies on the CAG repeat expansion in patients with hereditary spinocerebellar ataxia from Chinese Han. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2009, 26(6):620-5.

[12]. Jiang H, Tang B, Xu B, et al. Frequency analysis of autosomal dominant spinocerebellar ataxias in Han population in the Chinese mainland and clinical and molecular characterization of spinocerebellar ataxia type 6. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2005, 22(1):1-4.

[13]. Chen Z, Wang P, Wang C, et al. Updated frequency analysis of spinocerebellar ataxia in China. Brain. 2018, 141(4):e22.

[14]. Zhang X, Hao Y, Gu WH, et al. Genetics and clinical study of Chinese kindreds with dentatorubral pallidoluysian atrophy. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2013, 30(1):31-5.

[15]. Lee YC, Tsai PC, Guo YC, et al. Spinocerebellar ataxia type 36 in the Han Chinese. Neurol Genet. 2016, 2(3):e68.

[16]. Ouyang Y, He Z, Li L, et al. Spinocerebellar ataxia type 31 exists in northeast China. J Neurol Sci. 2012, 316(1-2):164-7.

[17]. Wang J, Shen L, Lei L, et al. Spinocerebellar ataxias in mainland China: an updated genetic analysis among a large cohort of familial and sporadic cases. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2011, 36(6):482-9.

[18]. Dong Y, Sun YM, Liu ZJ, et al. Chinese patients with Huntington's disease initially presenting with spinocerebellar ataxia. Clin Genet. 2013, 83 (4):380-3.

[19]. Fogel BL, Lee H, Deignan JL, et al. Exome sequencing in the clinical diagnosis of sporadic or familial cerebellar ataxia. JAMA Neurol. 2014, 71(10):1237-46.

[20]. Pyle A, Smertenko T, Bargiela D, et al. Exome sequencing in undiagnosed inherited and sporadic ataxias. Brain. 2015, 138(Pt 2):276-83.
[21]. Sun YM, Chen C, Wang J, Zhang Y. Clinical features and mutation characteristics of patients with Dentatorubral-pallidoluysian Atrophy in Chinese Han Population. Chin J Clin Neurosci. 2017, 25(2):183-90,200.

[22]. Zhou XY, Wu JJ, Sun YM. An atypical case of early-onset dystonia with a novel missense variant in KMT2B. Parkinsonism Relat Disord. 2019, 63:224-6.

[23]. Moro A, Munhoz RP, Arruda WO, et al. Spinocerebellar ataxia type 3: subphenotypes in a cohort of brazilian patients. Arq Neuropsiquiatr. 2014, 72(9):659-62.

[24]. Subramony SH, Schott K, Raike RS, et al. Novel CACNA1A mutation causes febrile episodic ataxia with interictal cerebellar deficits. Ann Neurol. 2003, 54(6):725-31.

[25]. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015, 17(5):405-24.

[26]. Samukawa M, Hirano M, Saigoh K, et al. PSP-Phenotype in SCA8: Case Report and Systemic Review. Cerebellum. 2019, 18(1):76-84.

[27]. Miyawaki T, Sekiguchi K, Yasui N, et al. A case of juvenile parkinsonism with expanded SCA8 CTA/CTG repeats. Rinsho Shinkeigaku. 2013, 53 (4): 278-82.

[28]. Owada K, Ishikawa K, Toru S, et al. A clinical, genetic, and neuropathologic study in a family with 16q-linked ADCA type III. Neurology. 2005, 65 (4): 629-32.

[29]. Serrano-Munuera C, Corral-Juan M, Stevanin G, et al. New subtype of spinocerebellar ataxia with altered vertical eye movements mapping to chromosome 1p32. JAMA Neurol. 2013, 70(6):764-71.

[30]. Németh AH, Kwasniewska AC, Lise S, et al. Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. Brain. 2013, 136(Pt 10):3106-18.

[31]. Ohba C, Osaka H, Iai M, et al. Diagnostic utility of whole exome sequencing in patients showing cerebellar and/or vermis atrophy in childhood. Neurogenetics. 2013, 14(3-4):225-32.

[32]. Sawyer SL, Schwartzentruber J, Beaulieu CL, et al. Exome sequencing as a diagnostic tool for
pediatric-onset ataxia. Hum Mutat. 2014, 35(1):45-9.

Figures
Figure 1
Flowchart of genetic testing.

Collecting autosomal dominant cerebellar ataxia patients

Screening dynamic mutations of SCA1,2,3,6,7,8,10,12,17,DRPLA

-ve

Screening dynamic mutation of HTT

-ve

Screening dynamic mutations of SCA31, 36

-ve

Performing whole exon sequencing (WES)
Figure 2

Results of the genetic testing and frequencies of SCA subtypes.

Figure 3

Pedigrees of family A-D with DRPLA. Arrow: proband; square: male; circle: female; slash: deceased; solid symbol: affected.