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

Fang Peng
Huashan Hospital Fudan University,Department of Neurology
Yue Zhang
Huashan Hospital Fudan University, Department of Neurology
Xin-Yue Zhou
Huashan Hospital Fudan University, Department of Neurology
Shuai-Qi Huang
Huashan Hospital, Fudan University, Department of Neurology
Chen Chen
Huashan Hospital Fudan University, Department of Neurology
Zheng-Tong Ding
Huashan Hospital, Fudan University, Department of Neurology
Jian Wang
Huashan Hospital Fudan University Department of Neurology
Yi-Min Sun
Huashan Hospital Fudan University, Department of Nuerology
Jianjun Wu (wujianjun@fudan.edu.cn)
Huashan Hospital https://orcid.org/0000-0001-6438-6334

Research

Keywords: autosomal dominant cerebellar ataxia, dynamic mutation, frequency, genetic testing, whole exome sequencing

Posted Date: February 17th, 2020

DOI: https://doi.org/10.21203/rs.2.23720/v1

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 ethnics 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	-	Unstable walking, died at the age 75
	II:4 (the proband)	68	69	Unstable walking
Family B	I:1	NA	-	Unstable walking, died at the age 59
	II:1	30	-	Unstable walking, died at the age 39
	II:2 (the proband)	30	45	Epilepsy, unstable walking
	III:2	19	22	Epilepsy, unstable walking
Family C	II:1	50	-	Unstable walking, died at the age 69
	III:1 (the proband)	39	44	Unstable walking, involuntary movement, irritability
	III:2	< 15	-	Epilepsy, unstable walking, dysphonia, bed-ridden, died at age 22
	III:5	30	-	Epilepsy, unstable walking, cognitive impairment, irritability, died at age 43
	III:7	30	33	Epilepsy, unstable walking, cognitive impairment
	III:8	25	30	Epilepsy, unstable walking
	III:9	23	24	Epilepsy, unstable walking, cognitive impairment from youth
	IV:2	Youth	21	Cognitive impairment
	IV:3	21	26	Epilepsy
	IV:4	Youth	23	Cognitive impairment, irritability
Family D	II:1	Youth	77	Unstable walking
	II:2	Youth	-	Unstable walking
	II:3	Youth	72	Unstable walking
	II:4	Youth	63	Unstable walking, 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, slurred speech, cognitive impairment
	IV:1	10	-	Cognitive impairment
	IV:2	5	-	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[24] was revealed, which has not been reported and according to the American College of Medical Genetics and Genomics (ACMG) guideline[25], 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 & Transcript	Variant & Protein	Het/ Hom	Mutation Taster prediction	Phoyphen-2 prediction	SIFT score and prediction	Allele frequ in Ex
I	F	60	61	Unstable walking, slurred speech	SLC1A3 (NM_004172.4)	c.397A > G & p.Ile133Val	Het	Disease causing	Benign	0.46 Tolerated	0.004
II	F	44	48	Unstable walking, slurred speech, bucking, blurred vision, horizontal nystagmus, tremor in hands, limbs ataxia	OPA1 (NM_015560.2)	c.598_603del	Het	Polymorphism	NA	NA	NA
III	M	45	46	Unstable walking, slurred speech, limbs ataxia, hyperreflexia	TTBK2 (NM_173500.3)	c.1359A > C & p.Lys453Asn	Het	Disease causing	Benign	0.00 Deleterious	NA
IV	F	59	62	Unstable walking, slurred speech, positional tremor	TTBK2 (NM_173500.3)	c.7G > C & p.Gly3Arg	Het	Disease causing	Probably damaging	0.00 Deleterious	NA
V	F	55	56	Unstable walking, weakness and numbness in bilateral lower limbs, legs cramp when sleep, hyperreflexia	TTBK2 (NM_173500.3)	c.3488C > T & p.Thr1163Met	Het	Disease causing	Probably damaging	0.00 Deleterious	NA
VI	M	46	47	Unstable walking, slurred speech, bucking, limbs ataxia, hyperreflexia in bilateral lower limbs, pain in right lower limb	CAMTA1 (NM_015215.2)	c.2191G > A & p.Gly731Ser	Het	Polymorphism	Benign	0.00 Deleterious	0.001
VII	M	50	52	Difficulty in walking, spastic gait, hyperreflexia, hypermyotonia, bilateral positive pathologic signs, normal MRI and EMG results	/	/	/	/	/	/	/
VIII	F	46	42	Unstable walking, slurred speech, bucking, limbs ataxia, hyperreflexia, serious decline in vision, ptosis, limited eye movements, macular pigmentation disorder, optic nerve fiber layer atrophy	/	/	/	/	/	/	/

Patient No.	Gender	Onset age	Current age	Clinical symptoms and signs	Gene & Transcript	Variant & Protein	Het/ Hom	Mutation Taster prediction	Phoyphen-2 prediction	SIFT score and prediction	Allele frequ in Ex
IX	M	51	42	Unstable walking, spastic-ataxia gait, hyperrefleixa, hypermyotonia, 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;

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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%[30] and 15%[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[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 neo applicable to this article as no datasets were generated or analyzed during the current study.

Funding

This work has no funding to support.

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.

Acknowledgements

We sincerely appreciate the patients and their family members for their help and willingness in this study.

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Figures

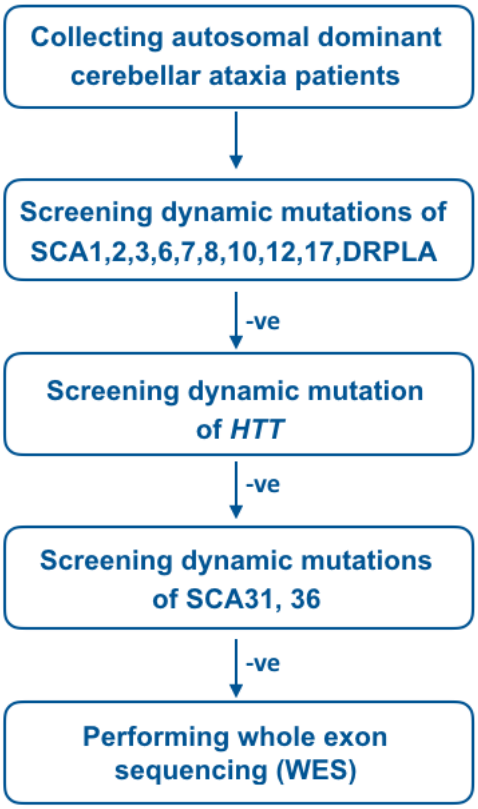

Figure 1

Flowchart of genetic testing.

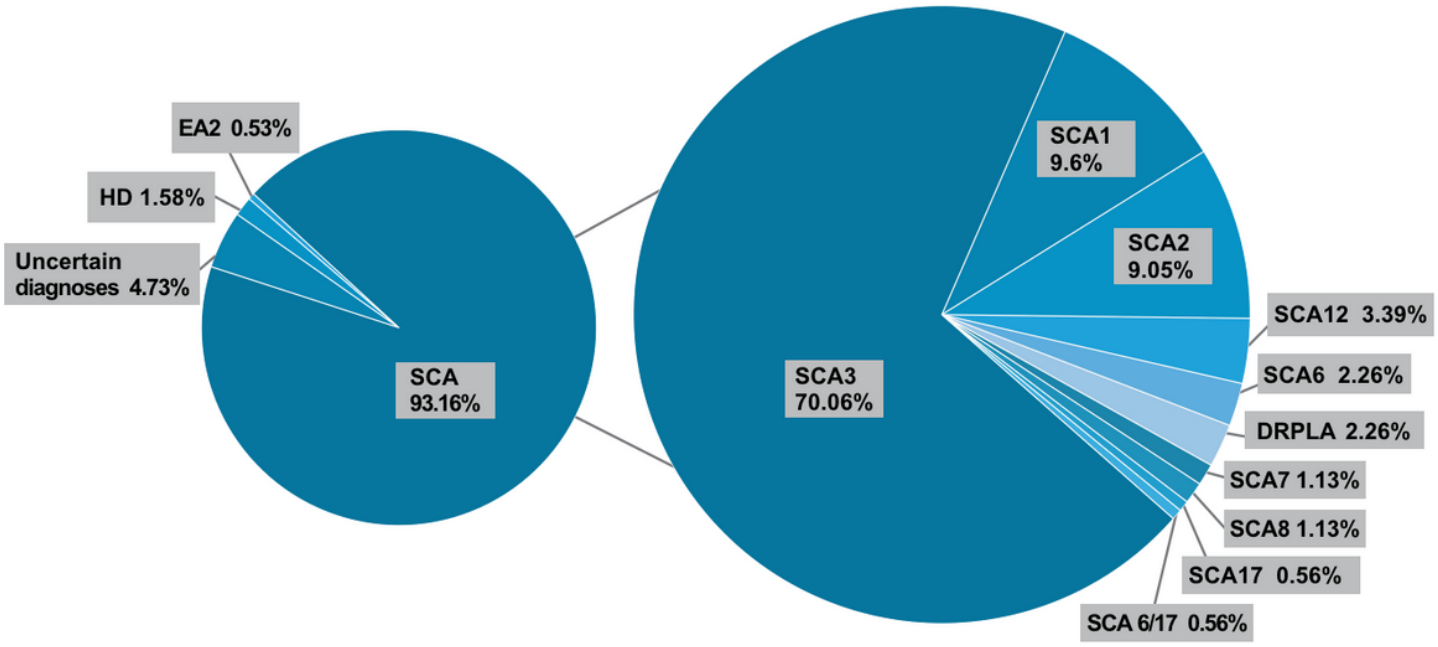

Figure 2

Results of the genetic testing and frequencies of SCA subtypes.

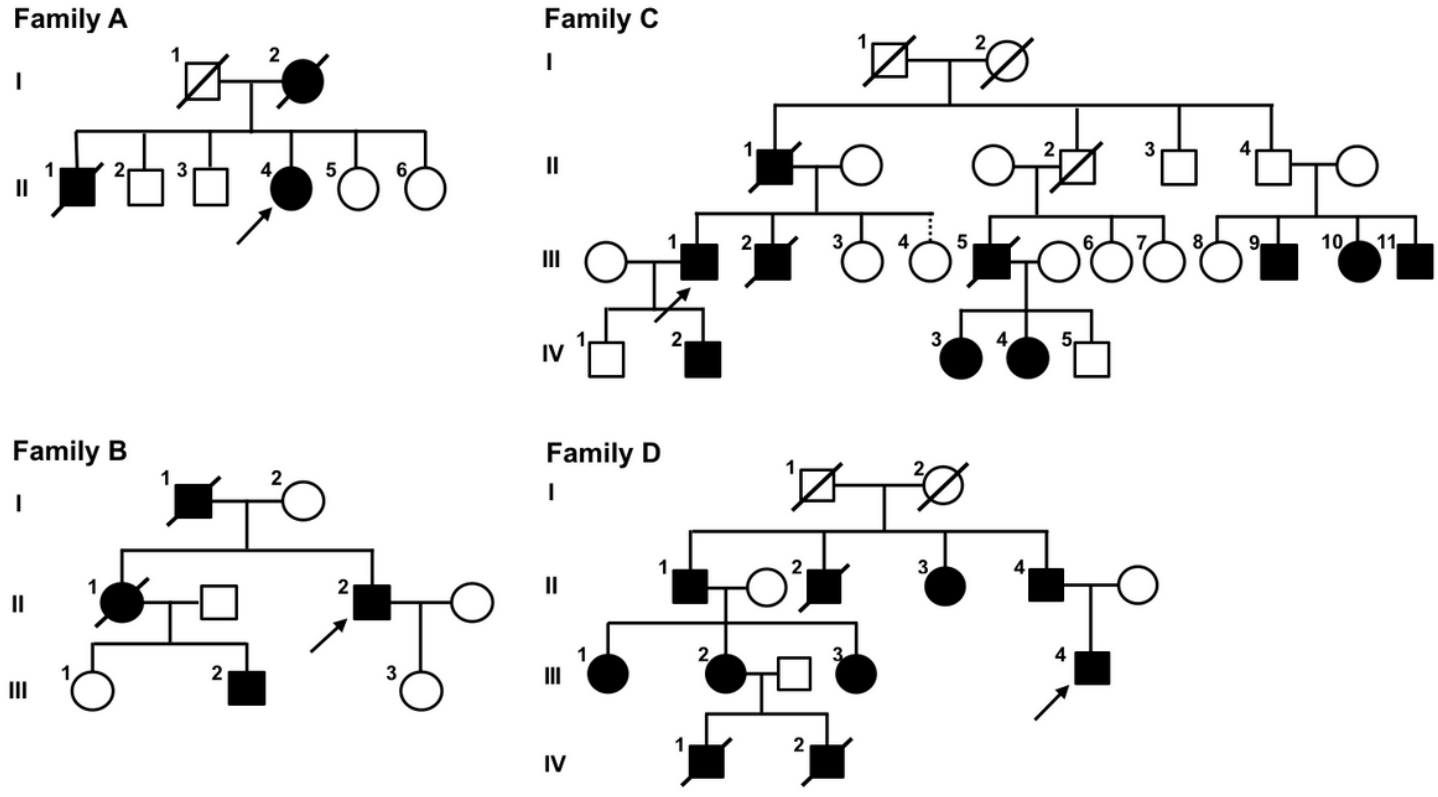

Figure 3

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