Early-onset familial essential tremor is associated with nucleotide expansions of spinocerebellar ataxia in China

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Research Article

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Abstract

Background

Essential tremor (ET) is a neurological disease characterized by action tremor in upper arms. Although its high heritability and prevalence worldwide, its etiology and association with other diseases are still unknown.

Method

We investigated 10 common spinocerebellar ataxias (SCAs), including SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, SCA36, dentatorubral-pallidoluysian atrophy (DRPLA) in 92 early-onset familial ET pedigrees in China collected from 2016 to 2022.

Result

We found one SCA12 proband carried 51 CAG repeats within *PPP2R2B* gene and one SCA3 proband with intermediate CAG repeats (55) with *ATXN3* gene. The other 90 ET probands all had normal repeat expansions.

Conclusion

Tremor can be the initial phenotype of certain SCA and it is necessary to screen SCAs in ET patients, especially in early-onset and familial patients.

1 Introduction

Essential tremor (ET) is a progressive neurological disease whose prevalence increases with age. The most characteristic feature of ET is its abnormal action tremor of upper arms, which includes kinetic tremor (i.e., tremor occurring in voluntary activities like eating or writing) and postural tremor (i.e., tremor occurring in resisting gravity motionlessly) [1]. In most patients, the disease progression is slow, and no definite therapy is found. It is noted that the phenomenon of familial aggregation is more prevalent in ET than in other neurological diseases, which implies that the genetic cause may contribute to ET [2, 3]. However, although some researchers have found genetic genes in large ET families and genome-wide association studies, few get reaffirmed in replication studies [4, 5].

Recently, studies have found that some ET patients got the wrong diagnosis because of the solo and mild symptom of tremor at the beginning [6–10]. Previous pathological studies have also found some overlaps in cerebellar changes between ET and other neurological diseases, such as spinocerebellar ataxias (SCAs) [11–13]. The correlation between the genotype and phenotype of SCAs remains ambiguous. Although ataxia is the most characteristic clinical feature in SCAs, some studies found certain subtypes could manifest tremor like ET [14–16]. The phenotypes between different races and lineages can also be

heterogeneous. However, when researchers investigated related SCA genes in suspected ET patients, the results were mostly negative [7, 17–20].

Since SCA prevalence is varied in different regions and the young-onset ET cases are more prone to be genetic factors [3], we selected early-onset Chinses ET patients with familial history to investigate the significance of screening SCA genes in routine diagnosis and treatment.

2 Patients and Method

A total of 92 patients from eastern mainland China were enrolled from 2016 to 2022 in the Fourth Affiliated Hospital and the Second Affiliated Hospital, Zhejiang University School of Medicine. The patients were diagnosed with ET based on the latest guideline [21] by senior movement disorder neurologists. Detailed neurological examination was done to exclude other diseases, such as Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and multiple system atrophy (MSA). The routine brain MRI scan was to exclude structural cerebellar lesions. According to patients' recall, we collected the onset age when the tremor appeared. Every participant manifested tremor before 45 years old, which we defined as "early-onset" [22]. Comprehensive demographic and clinical information were collected using questionnaires to ensure no participants were exposed to medications or pesticides that may damage the cerebellum. The Tremor Research Group Essential Tremor Rating Assessment Scale (TETRAS) was used to evaluate the severity of tremor, and the Mini-mental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA), were used to assess patients' cognition level. Apart from patient, the existence of at least one more affected individual in two consecutive generations was defined as dominant inheritance. The blood samples were restored in standard tubes after obtaining written consent.

The medical ethics committee of the Fourth Affiliated Hospital and the Second Affiliated Hospital, Zhejiang University School of Medicine, China, approved the study.

2.2 Genotyping

We extracted DNA from peripheral blood cells as per standardized protocol. Then the PCR was carried out under optimized conditions: 36 cycles consisting of two cycles of 30 s at 95 ℃ and 30 s at 70 ℃, two cycles of 30 s at 95 ℃ and 30 s at 65 ℃, two cycles of 30 s at 95 ℃ and 30 s at 60 ℃, and 30 cycles of 30 s at 95 ℃, 30 s at 56 ℃, and 30s at 72 ℃ preceded by 10 min at 95 ℃ and followed by 10 min at 72 ℃. The repeat expansions in SCA1 (*ATXN1*), SCA2 (*ATXN2*), SCA3 (*ATXN3*), SCA6 (*CACANA1A*), SCA7 (*ATX7*), SCA8 (*ATXN8*), SCA12 (*PPP2R2B*), SCA17 (*TBP*), SCA36 (*NOP56*), and DRPLA (*ATN1*) were calculated using capillary electrophoresis in an automated ABI PRISM 310 genetic analyzer (Applied Biosystems, Inc., Hangzhou) and GenScan analysis software (Applied Biosystems, Inc.).

3 Results

In total, we collected 92 ET patients (54 males and 38 females) to perform an analysis of repeat expansion sizes in ten SCA gene loci. The mean age at tremor onset of patients was 27.57 ± 10.09 years old and the mean disease duration was 11.2 ± 9.35 years. Compared to intermediate and pathogenic ranges [20], we identified one SCA12 proband carrying 51 CAG repeats and one SCA3 proband carrying intermediate CAG repeats (55). The normal ranges of the SCA genes in our patients were as follows: 22–31 (SCA1), 11–28 (SCA2), 14–43 (SCA3), 4–16 (SCA6), 5–11 (SCA7), 17–38 (SCA8), 2–25 (SCA12), 27–39 (SCA17), 3–9 (SCA36), 7–34 (DRPLA).

Patients.

The Proband (Fig. 1, Patient :5) in Family 1 is a 52-year-old woman who was otherwise healthy when, at the age of 44, she first discovered the tremor in her head. She didn't note it until she found the tremor in her arms 3 years later. Then she went to the local hospital where she was diagnosed with Parkinson's disease and was treated with levodopa. However, her tremor was not relieved and showed a worse trend with larger amplitude and longer tremor in her head and arms. Then she was diagnosed with ET in another hospital and was treated with propranolol. The tremor improved during the first year but showed no significant benefit with an increased dosage after that. She developed a broad-based, staggering gait and mild dysarthria during at that time. One year later, she showed mild dysphagia. Now she requires a walker and assistance for most of her daily activities. Neurologic examination of the proband was notable for her kinetic and postural tremor of the head and arms. Her muscle tone was elevated in both legs, and her tendon reflexes were brisk. Both Babinski signs were positive. The nose-to-finger tests were unstable, and the Romberg sign was positive too. The cognitive test was normal. Biochemical tests were normal, including ceruloplasmin level, thyroid function, and tumor markers. A brain MRI at 50 years old revealed a few ischemic lesions in bilateral lateral ventricles. It was worth noting that her brother (Fig. 1, Patient :2), mother (Fig. 1, Patient :4), maternal uncle (Fig. 1, Patient :1), and grandpa (Fig. 1, Patient :2) have similar symptoms. After we identified her real cause was SCA12 (10/51 CAG repeats), we tested the repeat expansion of *PPP2R2B* gene in her affected brother (Fig. 1, Patient :2) and asymptomatic sister (Fig. 1, Patient :4). It turned out that her brother was also a SCA12 patient (13/49 CAG repeats), and her sister's result was normal (10/10 CAG repeats).

The proband (Fig. 2, Patient :1) is a 43-year-old right-handed man who developed the tremor in his upper arms at age 23. Since then, the frequency and amplitude of tremor in his hands appeared more severe, which made it difficult for him to use spoons, hold plates, write, and engage in social activities. Especially when he felt anxious or stressed out in some situations, the tremor became more uncontrollable. While after moderate consumption of alcohol, it could get some relief. Being still or during sleep, the tremor disappear. One year ago, he found tremor in his head and began to take propranolol 50mg twice daily under the doctor's advice. However, the treatment didn't show significant benefit as the tremor still existed and showed a worse tendency. Neurologic examination was notable for his marked action tremor involving in upper arms and head with TETRAS score of 48. His finger-to-nose testing was abnormal, but nystagmus, dysarthria, and gait abnormalities were not found. His muscle strength was normal, and muscle tone was not elevated. The reflexes could be elicited, but no sign of Babinski's sign. The cognition

was within the normal range, based on the MMSE score of 30/30, and the MoCA score of 29/30. The brain MRI showed a few lacunar ischemic lesions but no cerebellar atrophy. Analysis of leukocyte DNA confirmed the intermediate CAG expansion of the SCA3 gene (14/55 CAG repeats). According to the patient's memory, his father (Fig. 2, Patient 6) died at 50 because of a traffic accident and didn't manifest tremor. However, the paternal uncle of the proband (Fig. 2, Patient :4) had a similar tremor manifestation and long disease duration. Regretfully, his uncle declined a neurologic examination and genetic testing.

4 Discussion

In our study, we screened 10 common SCAs in early-onset familial ET patients and found one SCA12 with pathological CAG repeat expansion and one SCA3 with intermediate repeat expansion. Other participants didn't carry abnormal repeat expansions in this research.

Action tremor has been proven to be highly presented in SCA12 and is often the earliest manifestation of the disease [14, 23]. The cerebellar signs in SCA12 patients are usually less prominent and disabling than those observed in other SCAs, which leads to a higher rate of misdiagnosis [8, 14, 24]. Our patient (Fig. 1, Patient :6), at first, had the classic phenotype of tremor of the head and arms, and later the cerebellar signs, such as ataxia, dysarthria, became more significant. However, not every SCA12 has the same harbinger. Dong et al [8] identified three SCA12 probands among 29 SCA index patients and found only one patient with his family initially presented with tremor, while the others exhibited gait ataxia from the beginning. Besides, the unstable expanded alleles in our study are also consistent with former studies [25, 26], which may explain the family's anticipation and heterogenous clinical features.

SCA3 was the most frequent form of SCA worldwide [27], and also in China [28]. The tremor in SCA3 mostly accompanies by parkinsonian symptoms, like bradykinesia, and is responsive to levodopa treatment [29, 30]. The isolated action tremor like ET was rarely reported [31]. The patient (Fig. 2, Patient :1) in our research harboring intermediate (55) CAG repeat lengths showed only progressive tremor of his arms without other neurological signs, such as ataxia. Previous reports also described SCA3 patients with intermediate repeats involved with peripheral nervous system dysfunction [32–35], like restless legs syndrome, and sensory axonal neuropathy. One assumption in explaining the mild performance of our patient is that only one intermediate allele is not enough to trigger polyglutamine-derived cytotoxicity compared with two intermediate alleles together. Besides, although the intermediate range from 40 to 63 is commonly agreed [20], there are still many controversies surrounding the range of CAG repeats of *ATXN3*. For example, Gan et al detected CAG repeats in 1003 Chinese population using the novel CAG repeat ladder and redefined the range as: intermediate alleles 45–49, and expanded alleles ≥ 50 [36]. Considering the different range cited by recent studies and the penetrance of the gene abnormality at a young age may not be 100% [37], we cannot rule out the possibility of a pre-symptomatic state in our patient. The follow-up and pedigree analysis will be necessary and needed.

Although two of our patients didn't show cerebellar changes in brain MR, the structural changes in the cerebellum have been identified in many post-mortem studies of ET and SCA [11, 12, 38, 39]. Converging lines of evidence have supported the presence of the tremor-related brain network, which consists of brainstem nuclei, cerebellum, thalamus, and motor cortex. The overlapping pathological changes between ET and SCAs are mostly centered on the Purkinje cell (PC) and neighboring neuronal populations [13,40–43]. It is already known that PC expresses glutamate receptor delta 2 (GluRδ2) and P/Q type voltage-dependent calcium channel (Cav2.1) while climbing fibers (CFs) synapses express vesicular glutamate transporter type 2 (VGlut2) and parallel fibers (PFs) synapses express VGlut1 [44]. The proper distribution of CFs and PFs along PC dendritic is essential for the excitatory inputs to PCs. In ET, the insufficiency of GluRδ2 protein, synaptic pruning dysfunction of CF-to-PC synapses, and cerebellar oscillations have been confirmed by autoptic cerebral tissue, clinical data, and animal models [45, 46]. While the cascade dysfunction of calcium signaling related to CF, including metabotropic glutamate receptors (mGluRs) [47], inositol 1,4,5-trisphosphate receptor 1 (IP_3R1) [48, 49] have been found in SCA subtypes. It is presumed that there is a common pathway in the pathogenesis of ET and SCA, and the only distinguishing feature lies in the degree of changing patterns [12]. However, as the phenomenon of excessive CF-PC synapses by increasing number of CF synapses stretching to the PF synaptic territories is exclusively expressed in ET, we cannot conclude that ET patients are predisposed to develop SCA. Besides, animal models can't completely duplicate human diseases, more research is necessary to deepen our understanding of the mechanism in the future.

Compared to other studies, the positive rate in our study is higher than in previous studies. Nicoletti et al first didn't find SCA12 in 30 familial ET in Italy [18]. Later, Tan et al found one SCA3 patient presenting like ET initially by screening the SCA3 gene in 177 ET patients [7]. Chen et al screened *PPP2R2B* CAG repeat length in 132 ET patients in the Han Chinese in Taiwan, but all were in the normal range [19]. Louis et al analyzed 10 common SCAs loci in 323 ET patients in the US, and no intermediate or pathogenic range of repeat expansions was found [20]. The reasons can be followed. First, most studies selected patients without considering the onset age, genetic background, and family history, which may be less likely to have a gene-related disease. Then, previous studies only focused on certain SCA subtypes, which could leave out suspected patients. Also, studies were mostly carried out in western countries rather than in China. Since the prevalence of the SCA subtype is different across countries, our research is necessary.

Some limitations in our work deserve mention. First, although we have tested 10 SCAs in this study, there are still other SCAs not included because of their rarity. Second, we collected the onset of tremor based on patients' memory and defined it as the onset age of the disease. This may cause recall bias, and whether the nonmotor performance earlier than the tremor could be a sign of ET is unknown.

5 Conclusion

In summary, we identified two SCA patients in 92 early-onset, familial ET individuals. Our study is in line with recent studies [9, 10] that ET may not be a solo disease, and follow-up visits of suspected patients

may be necessary. Notably, subtle signs of ataxia should be carefully examined when meeting these patients.

Abbreviations

ET: essential tremor; SCA: spinocerebellar ataxia; DRPLA: dentatorubral-pallidoluysian atrophy; TETRAS: The Tremor Research Group Essential Tremor Rating Assessment Scale; MMSE: Mini-mental State Examination; MoCA: Montreal Cognitive Assessment; PC: Purkinje cell; GluRδ2: glutamate receptor delta 2; Cav: voltage-dependent calcium channel; CF: climbing fiber; VGlut: vesicular glutamate transporter; PF: parallel fiber

Declarations

Ethical Approval

This study has been approved by the medical ethics committee of the Fourth Affiliated Hospital and the Second Affiliated Hospital, Zhejiang University School of Medicine, China. The written informed consent has been obtained from all patients involved in this work. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Competing interests

The authors have no conflicts of interest to declare.

Author's Role

Zhilin Zheng: study conceptualization, data analysis and writing original draft; Zeyu Zhu, Chen Zhou, Jinyu Lu, Dayao Lv, and Gaohua Zhao: data acquisition and data analysis; Xinzhen Yin, Jun Tian, and Yanxing Chen: project administration, supervision and review; Jiali Pu, Baorong Zhang and Yaping Yan: project administration, supervision, review, and editing; Guohua Zhao: project administration, funding acquisition and review.All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

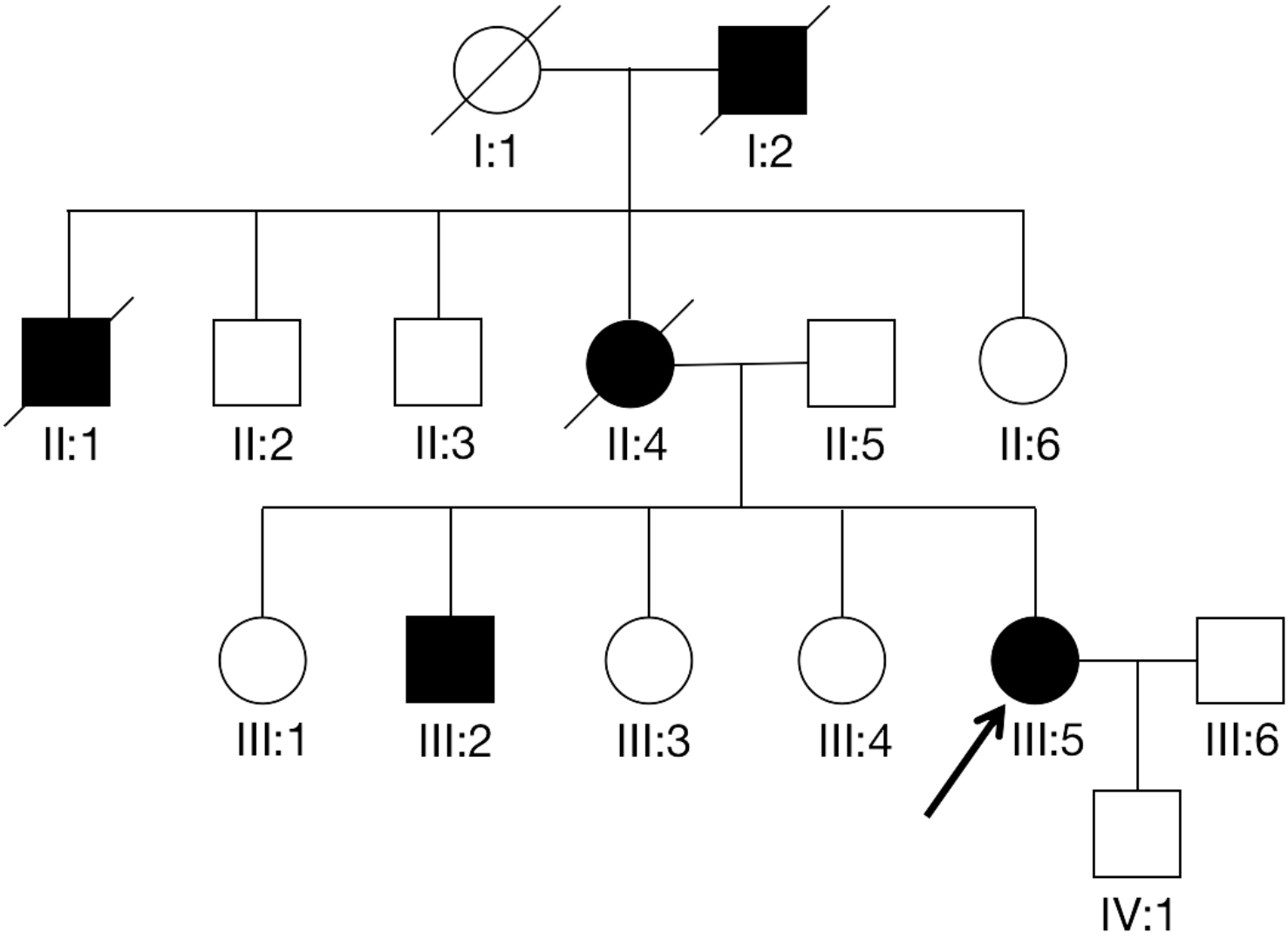

Figure 1

Pedigree of the SCA12 family. The arrow (Patient :5) indicates the proband

Circles = females; squares = males; black symbols = affected individuals; slashed symbols = deceased family members

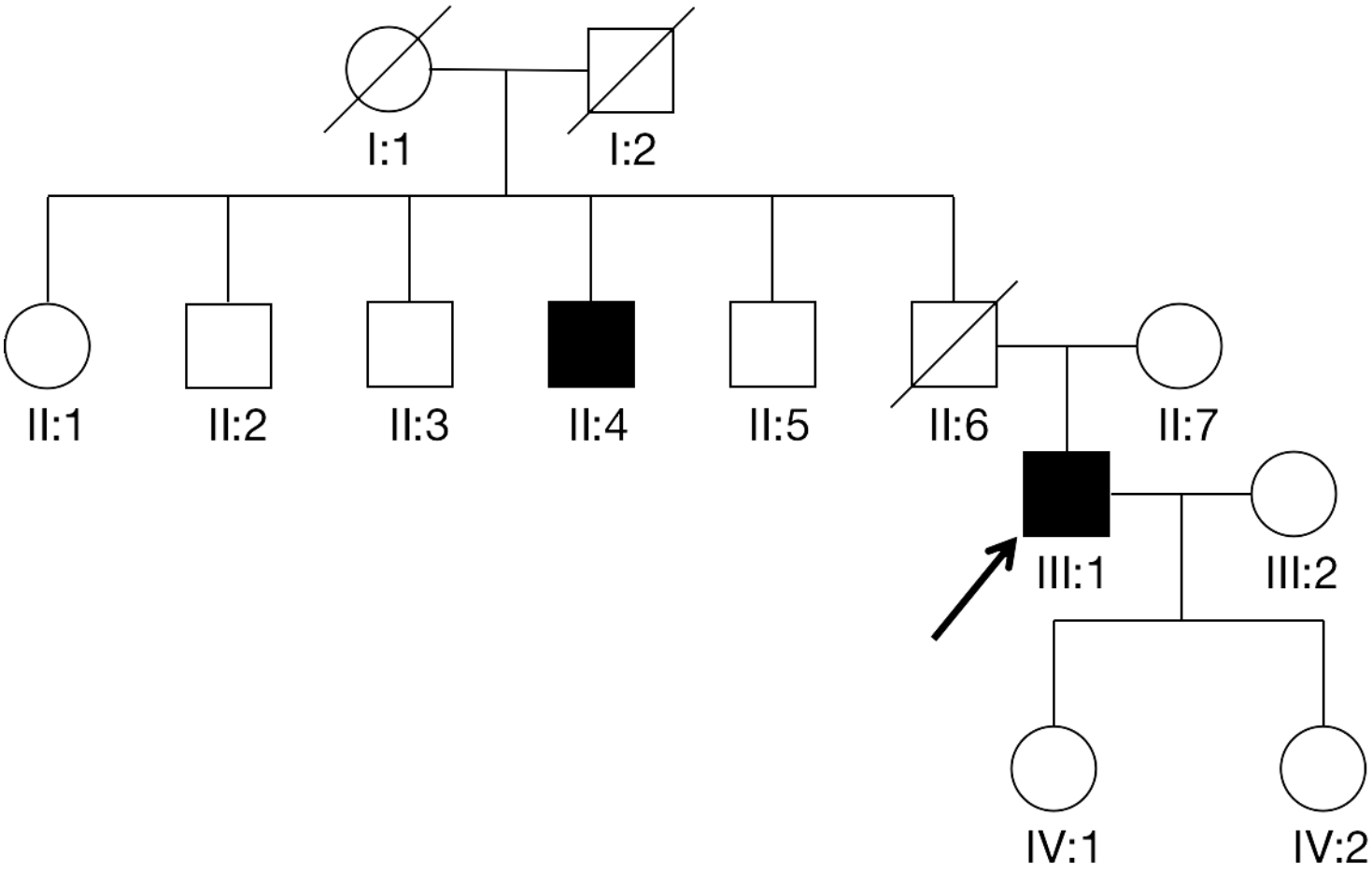

Figure 2

Pedigree of the SCA3 family. The arrow (Patient :1) indicates the proband

Circles = females; squares = males; black symbols = affected individuals; slashed symbols = deceased family members