

Determination of Genotypic and Phenotypic Characteristics of Friedreich's Ataxia and Autosomal Dominant Spinocerebellar Ataxia Types 1, 2, 3, and 6

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ABSTRACT

Introduction: This study aimed to analyze the genotypic characteristics of Friedreich's ataxia (FA) and autosomal dominant ataxias [such as spinocerebellar ataxia (SCA) types 1, 2, 3, and 6] using molecular and biological methods in hereditary cerebellar ataxia considering both clinical and electrophysiological findings.

Methods: The study included 129 indexed cases, who applied to the neurology department and were diagnosed with hereditary cerebellar ataxia through clinical, laboratory, and electrophysiological findings, and 15 sibling patients who were diagnosed through family scanning (144 cases in total); their genetic analyses were also performed. Detailed physical and neurological examinations, pedigree analyses, electroneurography, evoked potentials, cerebral–spinal magnetic resonance imaging, and echocardiographic analyses were performed for all cases. Blood samples were collected from patients, and the genotypic

characteristics of autosomal dominant SCA types 1, 2, 3, and 6 were investigated. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc; Chicago, IL, USA) 17.0.

Results: Almost 50% of patients were defined as FA. Moreover, two SCA1 cases and one SCA6 case were detected.

Conclusion: In our study, 47.2% of patients with FA had developed hereditary cerebellar ataxia. Ground and autosomal dominant-linked SCA1 and SCA6 were each detected in one family. These data suggest that patients with cerebellar ataxia of hereditary origin should be primarily examined for FA.

Keywords: Hereditary, Friedreich's ataxia, autosomal recessive ataxia, spinocerebellar ataxia

INTRODUCTION

Ataxia is defined as the loss of balance and coordination because of cerebellum and its connections, spinal cord damage, or peripheral sensorial losses. Ataxias may be classified as congenital, hereditary, non-hereditary, and symptomatic (1). Hereditary ataxias may be autosomal dominant, autosomal recessive, or X-linked according to Mendelian inheritance (2).

Spinocerebellar ataxias (SCA), inherited as autosomal dominant (AD), represent a neurodegenerative disease group that has a genetically and clinically heterogeneous structure. In studies performed in certain geographical regions, SCA prevalence has been found to be 3/100,000 individuals (3,4). As clinical findings of SCA subtypes greatly overlap each other and as different clinical pictures may be observed even in each SCA subtype, it is difficult to diagnose SCA only on the basis of clinical characteristics (3). Therefore, determining the genetic etiologies of SCA is very important for identifying and classifying this disease group (2). The Human Genome Naming Committee has currently identified 31 chromosomal loci and genes from SCA1 to SCA37 (5,6,7,8,9,10). The most common SCA types worldwide are 1, 2, 3, 6, and 7 (11,12), and cytosine–adenine–guanine (CAG) trinucleotide repeats are observed in these most common subtypes. Because of increases in the CAG trinucleotide repeats in the coding regions of relevant genes, polyglutamine products accumulate in the cell, resulting in neuron damage. These degenerated neurons containing increased polyglutamine levels have been most commonly found in the cerebellum, brain stem, and medulla spinalis (13).

In ataxias that are inherited as autosomal recessive (AR), in many families, only one member is sick. Typically, parents do not exhibit any symptoms as they are heterozygous (14). The risk of disease development in each child is 25% (1). Friedreich's ataxia (FA), being the most common AR-linked ataxia type, is caused by guanine–adenine–adenine (GAA) trinucleotide repeats in the first intron of the FRDA gene at the 9q21.1 position (15). FA represents 75% of all ataxias, which start before the age of 25 years (16,17,18,19). Re-increase in GAA repeat leads to a disorder in the synthesis of the frataxin protein, thus reducing mitochondrial respiratory activity, which paves the way for accumulation of free radicals in the cell (1,14). FA prevalence varies between 1/20,000 and 1/125,000 individuals (20). GAA repeats are inversely proportional to the age at disease onset (21,22).

In this study, the cases diagnosed with hereditary ataxia in light of history, neurological examination findings, and electrophysiological findings have been scrutinized in terms of FA and SCA types 1, 2, 3, and 6.



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METHODS

Following the approval of the Çukurova University Faculty of Medicine Ethics Committee, this study included 129 indexed cases, who were diagnosed with hereditary cerebellar ataxia in light of clinical, laboratory, and electrophysiological findings, and 15 sibling patients, who were diagnosed through family scans (144 cases in total). All cases were subjected to detailed history, pedigree analysis, physical examinations, and neurological examinations, and nerve conduction studies were performed using a Medelec Synergy electromyography device. Visual- (VEP), sensorial- (SEP), and brainstem-evoked potential (BAEP) studies were performed. In addition, cerebral-spinal MRG and echocardiographical examinations were also performed. In echocardiography, the left ventricular end-diastolic and -systolic volume calculations, ejection fraction, interventricular septum, and posterior wall thicknesses were measured.

Peripheral blood samples were obtained from patients, and DNA isolation was performed using a modified salting out method that was developed by Miller et al. (23). Cases were examined in terms of the existence of a frataxin mutation for FA, the existence of ataxin 1, ataxin 2, and ataxin 3, and voltage-dependent P/Q type calcium channel alpha 1a subunit (CACNA1A) gene mutations for SCA types 1, 2, 3, and 6. Moreover, 42 age- and sex-matched healthy volunteers were used as the control group.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS Inc; Chicago, IL, USA) 17.0 packet program was used for statistical analysis of data, and a chi-square test was used for intergroup comparisons of categorical measurements. The Mann-Whitney U test was used for comparing between two groups of measurement results that did not reveal a normal distribution, and the statistical significance level was 0.05 in all tests.

RESULTS

With respect to history and pedigree data, FA showed an AR inheritance pattern in 83 cases and an AD inheritance pattern in 61 cases. In 68 cases with FA showing the AR inheritance pattern, a molecular study revealed the GAA repeats in the gene coding for the frataxin protein. In two cases with FA showing an AD inheritance pattern, ataxin 1 gene mutation was found, while in two other cases, CACNA1A gene mutation was found.

Moreover, 68 cases were diagnosed with FA: 37 females (54.4%) and 31 males (45.5%). The average age of males was 20.1 ± 8.1 years and that of females was 19.06 ± 6.5 years; the overall average age was 19.8 ± 7.2 (range 2–34) years. In the FA group, no statistically significant difference was observed in patients in terms of age and sex ($p=0.761$ and $p=0.299$, respectively). In the FAA group, the average GAA trinucleotide increase was detected to be 677.7 times, and 54.4% of female cases and 45.5% of male cases were homozygous.

It was determined that a lack of balance was the main and first complaint in all groups. Neurological examination revealed cerebellar syndrome findings in all cases. In the manual motor power examination that was performed according to the Medical Research Council scale, evident weaknesses were observed in the lower extremity distals of 52.9% of FA cases, but no weaknesses were detected in SCA1 and SCA6 cases.

Mental retardation and optical atrophy were other neurological examination findings that distinguished FA cases from SCA1 and SCA6 cases, and these findings were detected in 14.7% and 16.1% of all FA cases, respectively.

Skeletal deformities were found in all three groups, but their varieties of pes cavus, mallet finger, scoliosis, and dome palate were a remarkable finding in FA cases.

In evoked potential studies, because of an assessment based on latencies and/or amplitudes, abnormalities were detected in SEP, VEP, and BAEP recordings of 37, 12, and 6 patients with FA, respectively, while all electrophysiological findings were normal in the other two groups.

In cerebral MRG examinations, a significant difference was detected between the groups in terms of cerebellar atrophy. In spinal MRG examinations, cord atrophy was detected in FA cases, while no cord atrophy was observed in the other two groups.

In echocardiography, abnormal findings were detected in four FA cases. Mitral valve prolapse was diagnosed in two of these four cases, mitral insufficiency in one case, and diastolic dysfunction in one case. Furthermore, in a cardiac Doppler study, the septum and posterior wall thicknesses, left atrium, left ventricle, end-systolic and end-diastolic volumes, and ejection fraction parameters were studied. The septum was found to be thick in FA cases, and no other difference was detected among the three groups in terms of other parameters.

Oral glucose tolerance tests, biochemistry panels, and hormone profiles were found to be normal in all three groups.

Table 1 comparatively summarizes the clinical and laboratory data from all three groups.

DISCUSSION

Hereditary ataxias are slowly progressing diseases inherited as AR, AD, and X-linked. Final diagnosis is based on molecular studies in hereditary ataxias. Clinical history, clinical examination, laboratory tests, and neuroimaging studies aid diagnosis.

In our study, in ataxia cases, which were considered to have developed on a genetic basis, both frequency and phenotypic characteristics of these forms were elucidated by considering the prevalence of FA and SCA types 1, 2, 3, and 6 in line with data from molecular studies.

FA was first described by the German doctor Nikolaus Friedreich in 1863, and AR is the most commonly observed form of inherited ataxias. In very broad terms, FA represents at least 50% of all hereditary ataxias. FA cases accounted for 47.2% of our study patients as well. FA arises from a FRDA (X25) gene mutation in the q13-q21.1 region of the 9th chromosome (24). In 96% of cases, because of increases in the GAA trinucleotide repeats that are localized in the first intron of the X25 gene, the frataxin protein controlling the quantity of iron in mitochondria is reduced (24). The greater the increase in the trinucleotide repeats, the earlier the age at disease onset and the heavier the clinical picture is. Pathological repeat count varies between 66 and 1700. Although the average GAA trinucleotide count of our cases was 677.7 (100–1300) times, no correlation was found between trinucleotide repeat count and the age at disease onset and disease severity ($p=0.278$).

Disease incidence is 1/30,000–50,000, and the carrier frequency is 1/60–1/10 (25,26). Clinical findings generally begin at the age of 5–15 years, and incidence before that ages of 20 years is an important characteristic of the disease (27). In our study, the disease onset age was 12.03 ± 3.9 years, which was found to be consistent with the literature.

In FA, clinical findings present themselves with progressive extremity and walking ataxia. In neurological examinations, in addition to cerebellar findings, weakness, deep sensory impairment, Babinski positivity, and loss of deep tendon reflexes (DTR) in lower extremities at an early stage are

Table 1. Clinical and laboratory findings of FA, SCA1 and SCA6 patients

	FA (n=68)	SCA1 (n=2)	SCA6 (n=2)
Onset age (years)	11.9±4.1	38	10
First and main complaint	Imbalance	Imbalance	Imbalance
Mental retardation	10 (14.7)*	-	-
Glance paresis	-	1 (50)*	-
Nystagmus	68 (100)*	1 (50)*	1 (50)
Optical atrophy	11 (16.1)*	-	-
Weakness	36 (52.9)*	-	-
Pyramidal (spasticity)	27 (39.7)*	1 (50)*	-
DTR			
Areflexia	43 (63.23)*	2 (100)*	2 (100)
Hyporeflexia	25 (36.77)*		
Pathological reflex	68 (100)*	1 (50)*	2 (100)
Deep sensory stimulus	68 (100)*	2 (100)*	2 (100)
Extrapyramidal signs	-	-	1 (50) (dystonia and torticollis)
Polyneuropathy	62 (91.1)*	2 (100)*	-
Skeletal deformity (pes cavus, mallet finger, and kyphoscoliosis)	68 (100)*	1 (50)*	2 (100)
Cerebral atrophy	-	-	-
Cerebellar atrophy	23 (33.8)*	-	2 (100)
Spinal atrophy	8 (11.7)*	-	-
Cardiac findings	4 (5.88)*	-	-
VEP stimulus	12 (17.6)*	N	N
SEP stimulus	37 (54.4)*	N	N
BAEP stimulus	6 (8.8)*	N	N
Diabetes mellitus	-	-	-
DTR: Deep Tendon Reflexes; FA: Friedreich's Ataxia; SCA1: Spinocerebellar Ataxia Type 1; SCA6: Spinocerebellar Ataxia Type 6; VEP: Visual-evoked Potential; SEP: Somatosensory-evoked Potential; BAEP: Brainstem Auditory-evoked Potential; N: normal. *(%), and n: number of patients			

among the basic characteristics of the disease. Skeletal deformities were determined in physical examination. In our cases, the findings started with imbalance and frequent falling complaints, and weakness was detected in 52.9% (paraparesis in 38.2% and quadriparesis in 14.7%) of the subjects. Deep tendon reflexes were considered lost and/or hypoactive in all of the cases. In almost all cases, besides Babinski positivity and/or indifference, deep sensory (basically vibrational sensations) stimulation was detected.

In FA, sensorineural hearing loss (8-13%), optical atrophy, and severe visual stimulation may be seen (17). In our cases, sensorineural hearing loss was not detected and optical atrophy was diagnosed in 16.1% of cases.

Cardiac findings (mitral valve prolapse, hypertrophic cardiomyopathy, systolic murmur, angina, arrhythmia, congestive heart failure, concentric ventricular hypertrophy, palpitation, hypokinetic dilated left ventricle, muscular subaortic stenosis) are other events that can accompany the findings for FA. In echocardiographical examinations performed on FA cases without any cardiac symptoms, increases in the intraventricular septum

and left ventricle posterior wall thicknesses and dilatation in left atrium were detected (28,29). These studies have demonstrated a correlation between GAA repeats and left ventricle hypertrophy (30). In 4 of our cases, echocardiographical findings characterized by mitral valve prolapse, 2° mitral failure, and diastolic dysfunction were determined. Furthermore, in tissue Doppler examinations, septum thickness was determined to increase in favor of FA cases ($p<0.05$).

Skeletal deformities (mallet finger, kyphoscoliosis, dome palate, pes cavus, pes planus, and echnivarus) are rather frequent. Milbrandt et al. (31) determined 63% had scoliosis and 24.5% had hyperkyphosis in a series of 77 patients. Previous studies have indicated that foot deformity prevalence was between 55% and 90% (32,33,34,35). In all of our cases, skeletal deformities consisting of one or more of such deformities (pes cavus, kyphoscoliosis, and dome palate) were detected.

Neuropathy and associated complaints are frequently reported in FA. In electrophysiological examinations, reduction inaction potentials was detected in the sensorial nerve or sensorial action potentials could not be obtained. Sensorial based mixed-type polyneuropathy findings were determined in 91.1% of our cases. Furthermore, though central transmission time has not been measured in our cases, due to peripheral or central stimuli, SEP, VEP, and BAEP abnormalities were detected in 54.4%, 17.6%, and 8.8% of our patients, respectively.

Vedolin et al. (36) have detected cerebellar atrophy in the MRG of patients and determined signal changes in the brain stem, cerebellum, and T1 and T2 weighted images. In addition, Akhlaghi et al. (37) have demonstrated that superior cerebellar peduncle atrophy is correlated to onset age, disease duration, and weight of clinical findings. From neuroimaging, cerebellar atrophy and spinal atrophy were determined in 33.8% and 11.7% of our FA cases, respectively, while cerebellar atrophy was diagnosed in the two SCA6 cases but spinal atrophy was not observed.

Though mental retardation is rarely reported in FA cases, mental retardation has been found in 14.7% of our cases (38). This percentage may also be correlated with consanguineous marriages (35.2%), which was rather frequent in our study. Furthermore, partial epileptic attacks have been observed in two of our patients (2.9%). These findings confirm the relationship of FA with mitochondrial cytopathies.

Considering not only FA cases described above, but also SCA1, SCA2, SCA3, and SCA6 cases studied together with laboratory tests, though there are exceptional examples, it was noted that the most commonly seen SCA type globally was SCA3 (39,40,41).

Other than the 68 FA cases inherited as AR, out of 61 cases considered to have been inherited as AD, two have shown the ataxin 1 (ATXN 1) (SCA1) gene mutation, and two have shown the CACNA1A (SCA6) gene mutation.

SCA1 is caused by ataxin-1 protein deficiency including an expanded CAG trinucleotide repeat coded by the ATXN 1 gene (42,43,44). Clinical findings have emerged in different decades such as ages 4-74 (generally in the 4th decade). Neurological examinations show cerebellar syndrome findings, oculomotor apraxia, slowing down in saccades, a reduction in vestibulo-ocular reflex, and reduction in optokinetic nystagmus. In nerve transmission studies, findings are characterized by sensorial or sensorial-motor axonal neuropathy. Pontine and cerebellar atrophy are detected in cerebral MRG. In our study, in the family considered to have SCA1, the clinical findings started at around the age of 40 years with imbalance and

speech disorders, and their neurological examinations revealed cerebellar findings, slowing down of saccadic eye movements, and impairments in the vibration sense.

SCA6 is correlated with a mutation in the voltage-dependent calcium channel P/Q type, alpha 1a subunit on the 19p13 chromosome (CACNA1A; CaV2.1). The CAG trinucleotide repeat is defined as normal between 4 and 18–20, and as ODSCA-6 between 20 and 33. Globally, its prevalence among SCA types is 13–15%. It is frequently seen in Germany, Taiwan, Australia, the United States of America, and Japan. Clinical findings are characterized by progressive cerebellar findings, and onset generally begins between the ages 19 and 71 (43–52) years (45,46,47,48). These findings may further be accompanied by not only vibration and proprioceptive sensorial loss, but also nystagmus (downward nystagmus), balance disorders emerging with head movements, and such extrapyramidal findings as dysphagia, bradykinesia, parkinsonism and dystonia, and eye movements may be restricted in all directions (45,46,47,48).

Neurological examination of male index cases of 17 years of age, diagnosed as SCA6 in our study, demonstrated cerebellar findings, optical atrophy, nystagmus, deep sensorial disorders, and corticospinal tracts stimulus findings, as well as extrapyramidal findings such as dystonia and torticollis.

In conclusion, hereditary ataxias are finally diagnosed by molecular genetic studies. Neurological examination findings, and laboratory tests consisting of electrophysiological and neuroimaging aid diagnosis and classification of ataxias. The duty of the clinician is to make a correct typing as far as possible, thereby minimizing the time and cost of molecular analysis studies.

It may not be possible to always obtain correct information from families and to draw a healthy pedigree. As also seen in our daily practice, patients, or their relatives, may try to deny or associate their diseases with individuals having neurological findings developing on hereditodegenerative grounds in other generation(s). By also considering the frequency of consanguineous marriages in our country, in the case of an ataxia, we must first evaluate in detail the FA phenotype and its associated findings. If FA diagnosis can be ruled out by the associated findings, we must consider AD inherited SCA and other causes of ataxia.

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