

## Mutation analysis of 6 spinocerebellar ataxia (SCA) types in patients from southern Turkey

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**Background/aim:** Spinocerebellar ataxias (SCAs) are complex clinical and genetically heterogeneous, mostly autosomal dominant neurodegenerative diseases. At present, more than 30 hereditary SCA types have been associated with different gene mutations. In this study, the frequency distribution of the 6 SCA types 1, 2, 3, 6, 7, and 17 in the Turkish population was investigated with respect to clinical features.

**Materials and methods:** 159 patients who received a diagnosis of SCA and 42 healthy controls from Adana, Mersin, Gaziantep, Hatay, and Osmaniye provinces were included in the study. DNA samples were isolated from 2 mL blood samples and the number of trinucleotide repeats (TNRs) for each SCA type was detected using PCR-RFLP technique and sequencing.

**Results:** Of the 6 SCA types that were studied, 4 types, SCA 1, 3, 7, and 17, were positive and all heterozygous for expansions. SCA types 1 and 17 had higher frequencies, 4.4% and 3.8%, respectively, than SCA types 3 and 7. The clinical data of patients were also evaluated to correlate with the increased TNR numbers.

**Conclusion:** This study, being the first mutation record of SCAs in this area, indicated that 9.4% of cases belonged to 4 types, SCA 1, 3, 7, and 17.

**Key words:** Spinocerebellar ataxia (SCA), trinucleotide (CAG) Repeat (TNR), PCR-RFLP

### 1. Introduction

Spinocerebellar ataxias (SCAs) are complex neurodegenerative diseases that are characterized by ophthalmoplegia, loss of vision, dysarthria, dementia, and loss of muscle coordination (1–3). SCAs are mostly dominantly inherited (1). Some types of this complex disease are starting to be defined with the discovery of different gene loci causing the syndrome (4). More than 30 types of SCA have been associated with distinct loci with the most common being types 1–3, 6, and 7 (5). So far, genes and mutations responsible for 15 of the SCAs have been identified by genetic studies and for the others, although association studies by linkage analysis have been completed, no specific genes and mutations have yet been identified (6).

Most of the mutations causing SCAs result from abnormal increases in the number of trinucleotide repeats (TNRs) (7). The amount of the increase in TNRs is thought to be related to the severity of the symptoms and with the age of onset of the disease, although no conclusive evidence has been found yet (8). At the moment, there is

no cure for the disease but correct identification of the type of disease is important for early diagnosis of the syndrome as it may occur in other family members as well as for the improvement of patient life quality.

The genes related to these types of ataxias normally encode a protein called ataxin and the CAG codon encodes glutamine amino acid. However, the affected gene with the increased CAG repetitions in the encoding region produces a mutant protein that contains far more glutamine residues than usual; therefore, these types of ataxia are classified as polyglutamine diseases (9). Mutant ataxin protein has a toxic function causing neurodegeneration (6).

Another complex neurodegenerative disease caused by trinucleotide expansion mutations that occur in the coding regions is Friedrich's ataxia (FA), which is known to have very similar symptoms to those of SCA. FA patients living in almost the same geographic area as those of this study were previously studied by Yilmaz et al. (10).

The SCA types studied here (1, 2, 3, 6, 7, and 17) are caused by CAG repeat expansion mutations in the coding regions of the related genes (6). Thus, in this study it is

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hoped that obtaining genetic information about the mutation ratios and the correlations among the number of TNRs, age onset, and severity of symptoms will assist early diagnosis, treatment, and genetic counseling for the population studied.

## 2. Materials and methods

### 2.1. Subjects and DNA isolation

The patients and control groups studied consisted of people living in Adana, Mersin, Gaziantep, Hatay, and Osmaniye provinces (southern Turkey) who presented to the Çukurova University Medical Faculty Hospital during 2007–2009; the patient group members presented to the Neurology and Pediatric Neurology Departments for various neurological complaints and were diagnosed as SCA; the control group members came to other departments for nonneurological complaints. Each patient or control was informed about the study before blood sampling and was asked to sign an informed consent form approved by the Ethics Committee of the Çukurova University Medical Faculty. Of the patient group consisting of 159 subjects clinically diagnosed with SCA and the control group consisting of 42 healthy people, each individual was separately tested for all 6 types of SCA genes: ATAXIN1, ATAXIN2, ATAXIN3, CACNA1A, ATXN7, and TBP. A blood sample of 2 mL was taken from each patient and control and placed into tubes containing EDTA; DNA samples were obtained using the salting out method (11).

### 2.2. PCR-RFLP and statistical analysis

Information about the complaints of the patients and other clinical and laboratory results were obtained from medical records prepared by the specialist neurologists who examined them. Regions with increased TNRs were amplified using a standard PCR amplification protocol (12). The primers used for PCR amplification are given in Table 1 (13,14).

The lengths of the PCR fragments amplified using the PCR-RFLP technique were determined using DNASIZE (15) following a 3% agarose gel run. This program enabled us first to measure the length of the product in the original gel run and then to calculate the increased number of sample TNRs. At first, the expected bp length of the product was accepted as standard (Table 1), i.e. 308 bp (16,17) for SCA 7 and 203 bp (18,19) for SCA 17 were subtracted and then divided by 3 to find the increased TNR numbers. After that, the expected reference TNR numbers of 10 for SCA 7 (16,17) and 38 for SCA 17 (18,19) were added to the increased TNR numbers to find the total TNR numbers.

The relationship between the TNR numbers and variables such as patient complaints, age, age of disease onset, and sex were analyzed using SPSS 13.0. Lastly, the

accuracy and validity of the agarose gel measurement method used here was tested by sequencing the related DNA regions, especially from the samples having expanded alleles (Table 2). Dye Cycle Sequence (Applied Biosystems) agents and a 3130 Genetic Analyzer (Applied Biosystems) were used for sequencing to determine the exact numbers of TNRs.

## 3. Results

For SCA types 1, 2, 3, 6, 7, and 17, the affected genes and the largest ranges of TNRs for normal, premutational, and expanded alleles cited to date are shown in Table 1. Each of the 42 healthy controls and 159 clinically diagnosed SCA patients was analyzed separately for increases in TNR numbers to distinguish SCA types 1, 2, 3, 6, 7, and 17. Ranges and averages of TNR numbers in the controls and patients studied and the relative frequency of the particular SCA types in all patients and controls are given in Table 2. When constructing a control group for a SCA type, people having no expanded allele for the related SCA type were considered as normal and, in that way, a SCA type-specific control cohort was assembled. The total number of a control cohort particular to a SCA type is supposed to be the sum of the healthy controls plus all the patients diagnosed as SCA except for the patients of the SCA type considered; the number of control cohorts therefore changed between 195 and 201 as the number of related SCA types were subtracted. Of the 6 SCA types studied, 4 types (SCA 1,3, 7, and 17) were observed with some percentages, with two of these (SCA types 1 and 17) having higher frequencies of 4.4% and 3.8%, respectively, and all 4 types having one allele expanded (i.e. all were heterozygous). When calculating the average TNR numbers shown in Table 2, three patients having the expanded allele at a premutation level were also included; these patients showed low penetrance and duly had mild symptoms. The other two types studied, SCA 2 and 6, were not encountered in the patients and none of the SCA types were found in the healthy controls (Table 2).

The primary complaints of patients are noted in Table 3. The age of onset of SCA in the patients ranged between 3 and 40 years old and the rate of consanguineous marriage was found to be considerably higher for SCA types 1 and 7 (Table 3).

## 4. Discussion

Repetitive DNA regions account for nearly 50% of the human genome (20) and changes in the repetitive DNA regions are thought to contribute to the diversity of many species during evolution (21). Mammals have developed various systems in order to prevent changes in the repeat numbers that can be harmful when a critical threshold value is exceeded for the repeat numbers. McMurray (22)

**Table 1.** Affected genes, primers used for PCR amplification, expected amplification length, and reference TNR ranges for SCA types 1, 2, 3, 6, 7, and 17.

Affected gene	Forward primer (5' → 3')	Reverse primer (5' → 3')	Expected size of PCR product (expected TNR number)	Reference TNR ranges	
				Normal	Pre-mutation Expanded
SCA 1 (13,14)	CAACATGGGCAGTCTGAG	AACTGGAATGTGGACGTAC	212 (29)	6-36	37-42 43-81
SCA 2 (13,14)	GGGCCCTCACCATGTCTG	CGGGCTTGGCGGACATTGG	130 (23)	16-30	31-34 35-59
SCA 3 (13,14)	CCAGTGACTACTTTTGATTTCG	TGGCCTTTCACATGGATGTGAA	202 (12)	12-44	45-65 66-80
SCA 6 (13,14)	CACGTGTCTATTCCCCCTGTGATCC	TGGGTACCTCCGAGGGCCGCTGGTG	141 (13)	4-20	- 21-27
SCA 7 (13,16,17)	TGTTACATTGTAGGAGCGGAA	CACGACTGTCCCAGCATCACTT	308 (10)	<17	17-36 37-460
SCA 17 (13,18,19)	GACCCACAGCCTATTTCAGA	TTGACTGTGAACGGGCTGCA	203 (38)	25-42	43-48 49-66

**Table 2.** Ranges of TNR numbers in controls (n = 42) and patients (n = 159) studied and relative frequency of SCA types in patients.

	n		TNR numbers (means $\pm$ SD)			Frequency of SCA type
			Control		Patients	
	Control	Patients	All alleles	Normal allele	Expanded allele	
SCA 1	194	7	10–33 (28.2 $\pm$ 2.3)	26–33 (29.7 $\pm$ 3.0)	43–58 (49.0 $\pm$ 5.5)	4.4%
SCA 2	201	0	20–28 (23.5 $\pm$ 1.8)	–	–	–
SCA 3	200	1	13–36 (16.3 $\pm$ 2.8)	18 (18.0 $\pm$ 0.0)	72 (72.0 $\pm$ 0.0)	0.6%
SCA 6	201	0	8–18 (12.7 $\pm$ 1.3)	–	–	–
SCA 7	200	1	9–17 (12.9 $\pm$ 2.4)	17 (17.0 $\pm$ 0.0)	77 (77.0 $\pm$ 0.0)	0.6%
SCA 17	195		30–42 (36.5 $\pm$ 3.4)	30–38 (32.8 $\pm$ 2.8)	45–63 (53.5 $\pm$ 8.0)	3.8%

**Table 3.** Clinical data of patients who had one of the alleles expanded for the related SCA type.

	n	Age at examination	Age at onset	Consanguineous marriage rate	Primary complaint
SCA 1	7	5–50	7–40	57.1% (n = 4)	Gait disorders
SCA 3	1	39	12	0% (n = 0)	Imbalance
SCA 7	1	5	3	100% (n = 1)	Loss of vision
SCA 17	6	3–44	1–36	0% (n = 0)	Imbalance

stated that for repeat numbers over 25–40 in the coding region of the gene and 55–200 in the noncoding region, simple TNR repeats become unstable and override the mechanisms that prevent the increase in TNR numbers and are prone to increase during an individual's life and in parent-child transitions.

Expansion mutations in the glutamine coding regions of these genes result in spinocerebellar ataxia because the mutant protein gains a new function as a result of polyglutamine increase (23), as is probably the case in the SCA type 1, 2, 3, 6, 7, and 17 gene regions examined in our study. Although it is still unclear, there are some opinions explaining how the increased polyglutamine in proteins causes ataxia. According to Gatchel and Zoghbi (24), a polyglutamine increase causes production of an abnormal toxic protein that builds up within the cell since the cell protein homeostasis is impaired. In sensitive cells like neurons, this has many harmful consequences such as abnormal function of organelles, defects in axonal conductivity, and defects in synaptic activity. According to Williams and Paulson (25), a polyglutamine increase also has an impact on the quality control mechanisms of the proteins and triggers the build-up of misfolded proteins in the cell. Although these and other similar views put forward are not conclusive, they all share the common idea of toxic effects of a polyglutamine increase in proteins.

It is known that the average TNR numbers vary according to geographical region and race. In this study, the average TNR numbers in genes with SCA types 1, 2, 3, 6, 7, and 17 and the frequency of each type relative to all SCA patients studied have been recorded for the first time for the Çukurova population of Turkey. To determine the TNR numbers, we used PCR-RFLP combined with DNASIZE (15). This fast and easy method was confirmed by the sequencing data of related genes as an adequate method since no differences were found between the results of the agarose gel electrophoresis and the sequencing.

Other clinical data of patients such as age of onset, mental retardation state, primary complaints, consanguineous marriage status, sex, and paresis state were also evaluated to correlate with the increased TNR numbers, but no statistically significant results were obtained, probably due to the small number of patients studied. Age of disease onset can vary from infancy to the age of 70; in our study it varied from 3 to 40 years of age and, as is generally accepted by many authors, age of disease onset is inversely correlated to the TNR numbers and patients with early onset are more likely to receive their TNR regions via transmission from their parents (26,27). It was also observed that the degeneration progresses much faster in patients with early onset (6). Several studies showed (28–30) that in SCAs with early onset in

childhood, the affected allele is transmitted to the child mostly from the father, and according to David et al. (31), the TNR numbers in the sperm cells of SCA patients are significantly higher than the TNR numbers in blood cells. It may also be noteworthy to state our observation on the patients with consanguineously married parents, whose alleles, even the normal alleles, tend to have higher averages of TNR numbers than the controls (Table 2). However, this assumption could not be examined in detail due to the lack of parental blood samples.

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It appears that this study includes the first mutation records of SCAs presenting a relative prevalence of 6 SCA types in 6 provinces of southern Turkey. The study indicates that 9.4% of cases belonged to 4 types: SCA 1, 3, 7, and 17.

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