Voice Alterations in Patients With Spinocerebellar Ataxia Type 7 (SCA7): Clinical-Genetic Correlations

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Summary: Background/Objectives. Spinocerebellar ataxia type 7 (SCA7) is an inherited neurodegenerative disease caused by the expansion of a cytosine-adenine-guanine triplet located in the coding region of the *ATXN7* gene, which is characterized by cerebellar ataxia, pigmentary macular degeneration, and dysarthria. Although dysarthria is a common feature in various SCA, its clinical characterization has been barely approached.

Patients/Methods. In this study, we report, to our knowledge for the first time, a detailed voice analysis in a large series of patients with SCA7, using different vocal parameters, including jitter, shimmer, and fundamental frequency. Patients were molecularly diagnosed using fluorescent-based polymerase chain reaction and capillary electrophoresis, and clinically characterized using the Scale for the Assessment and Rating of Ataxia and the Inventory of Non-Ataxia Symptoms.

Results. We found altered jitter, shimmer, and fundamental frequency measurements in patients with SCA7 compared with control subjects ($P < 0.05$). However, voice impairment was found unrelated with both age at disease onset and size of the cytosine-adenine-guanine triplet tract. Remarkably, jitter and shimmer measurements of patients were found to correlate with their Inventory of Non-Ataxia Symptoms, but not with their Scale for the Assessment and Rating of Ataxia scores, implying that voice impairment is the result of extra-cerebellar manifestations of the disease.

Conclusions. We propose that deficiency of the extra-cerebellar component of SCA7 might lead to sudden changes in laryngeal muscle tone, producing instability in sustained vowel phonation. Clinical characterization of voice will help to discriminate SCA7 from other SCA and to guide vocal therapy treatments.

Key Words: Spinocerebellar ataxia type 7 (SCA7)–CAG repeats–Shimmer–Jitter–Fundamental frequency.

INTRODUCTION

Spinocerebellar ataxia type 7 (SCA7) is an autosomal-dominant inherited neurodegenerative disease that is mainly characterized by progressive cerebellar ataxia and pigmentary macular degeneration.[1–3] SCA7 belongs to a growing group of genetic diseases caused by the expansion of unstable microsatellite repeats;[4] in particular, SCA7 was shown to be caused by an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat located in the coding region of the *ATXN7* gene, which mapped in the 3p12-21.1 chromosomal region.[5] The polymorphic tract of CAG repeats ranges from 4 to 18 repeats in a normal population, whereas affected individuals exhibited mutant alleles containing from 36 up to 460 repeats, which results in the incorporation of a segment of polyglutamines into the protein.[5] Interestingly, longer expansions that result in earlier disease onset and more severe symptomatology occur in subsequent generations of a given genealogy.[3,6] SCA7 is considered one of the rarest forms of autosomal-dominant inherited ataxias, with very few studies demonstrating clinical characterization of patients.[1–3] However, an increased number of SCA7 cases have been reported recently in various populations, including Swedish, Finnish, South African, and Mexican,[7–9] which would allow for a better description of the disease.

In addition to the cerebellar ataxia, SCA7 is often accompanied by other neurological features, including pyramidal symptoms, oculomotor disturbances, peripheral neuropathy, cognitive impairment, and sleep disorders, and could eventually develop more extensive neurological deficits that include dysphagia, hypoacusis, and dysarthria.[3,10] Alterations of the motor speech system that are associated with ataxic dysarthria include slow speaking rate, distorted consonant and vowel production, and impaired prosodic modulation of sentence utterances.[11,12] To date, acoustic voice analysis has been applied to describe the disordered voices of patients with any laryngeal pathology;[13–15] therefore, this analysis is essential to quantify the regularity and stability of vocal fold vibration.[16,17] However, to our knowledge, there are no data of its behavior in patients with SCA. Despite their clinical heterogeneity, dysarthria is a common hallmark for various spinocerebellar ataxias; however, characterization of the specific speech and voice alterations present in each SCA subtype has been scarcely approached.[11,18]

In this study, we report, to our knowledge for the first time, the clinical characteristics of the voices of a large group of patients with clinically and genetically characterized SCA, using an acoustic voice analysis, which allows us to associate voice alterations with the extra-cerebellar component of SCA7.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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METHODS

Subjects

The sample consisted of 33 subjects with SCA7, who were recruited from two different populations (Tlaltetela and Tuzamapan) of Veracruz State, Mexico,[19] and 33 unrelated healthy individuals from general Mexican population who were matched with patients by gender and age (control group). Patients with another SCA subtype or secondary ataxias (due to alcoholism, neoplasias, autoimmune or inflammatory diseases, vascular pathology, malformations, and other nongenetic causes), as well as patients with SCA7 who presented detectable hoarse voice on the recording day or with a history of voice disorder, were excluded. Patients were clinically assessed following Mayo Clinic procedures,[20] and by using two neurological rating scales: the Scale for the Assessment and Rating of Ataxia (SARA),[21] and the Inventory of Non-Ataxia Symptoms (INAS).[22] Signed informed consent was obtained from all study subjects, and the research protocol was approved by the Ethics and Investigation Committee of the National Institute of Rehabilitation (Mexico City).

SCA7 molecular diagnosis

Genomic DNA was extracted from peripheral blood leukocytes using the Gentra Puregene Blood Kit (Qiagen, Hilden, NRW, Germany). Fluorescent multiplex polymerase chain reaction (PCR) was performed on 60 ng of DNA template using the chimeric primers previously described.[23] Multiplex PCR was performed in a total volume of 6 μL containing 2.35 μM of each primer, 200 μM of each of the four dNTP, 0.6 μL of 10X reaction buffer, 2 mM of $MgCl_2$, and 0.5 U of Taq DNA polymerase (Roche Diagnostics GmbH, Mannheim, BW, Germany). PCR was conducted in a thermal cycler system (StepOne; Applied Biosystems, Foster City, CA, USA) as described previously.[9] Aliquots of PCR reactions were mixed with deionized formamide and internal size standard (GeneScan-500 TAMRA; Applied Biosystems), heated at 95°C for 7 min, cooled on ice for 5 min, and then electrophoresed through capillary on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Electrophoresis was carried out at 15 kV for 40 min at 60°C with 5 s of injection time. Genotyping data were analyzed with *GeneScan* software (Applied Biosystems, Foster City, CA, USA). Moreover, to discard false negatives, we performed TriPlet repeat-primed-PCR analysis as described by Cagnoli et al.[24]

Acoustic analyses

Participants were asked to "sustain /a/ for 5 s at habitual pitch and loudness." Recording was carried out in a quiet room with a recording microphone positioned at a distance of 10 cm from the subject's mouth, according to European and American assessment guidelines.[14,15] The procedure was repeated three times to obtain a total of 99 vowel phonations. Signal acquisition analysis was performed using *lingWAVES Clinical Speech and Voice Assessment* ver. 2.6 software (WEVOSYS, Forchheim, Germany). Each phonation was analyzed to obtain different perturbation measurements: shimmer (in %), jitter (in %), and fundamental frequency (in Hertz).

Statistical analysis

All results are expressed as mean ± standard error. Differences between patients with SCA7 and healthy individuals were determined by independent sample *t* test. We analyzed jitter, shimmer, and fundamental frequency data using the Student *t* test for unpaired samples (for parametric data and coefficient of variation <30%), whereas comparison of voice parameters between patients with early-onset disease and those with adult-onset disease with healthy subjects was performed employing one-way analysis of variance, followed by the Student standard Newman-Keuls *post hoc* analysis. Correlation between acoustic voice measurements and number of CAG repeats and SARA and INAS scale values was conducted using Pearson correlation coefficient (ρ). Significance levels were set at 95%. Statistical analysis was performed using *SPSS Statistics* 19 software (IBM, Armonk, NY, USA).

RESULTS

Patients with SCA7 and control subjects were molecularly diagnosed as described in the Methods section. Patients demonstrated mutant alleles ranging from 37 to 62 CAG repeats, whereas control subjects exhibited normal alleles ranging from 8 to 13 CAG repeats. Clinical features of patients with SCA7 are depicted in Table 1. All patients with SCA7 showed cerebellar syndrome, characterized by ataxia gait, dysarthria, intentional tremor in upper limbs, dysmetria, dysdiadochokinesia, hyperreflexia, and visual impairment. Other symptoms commonly found include slowing saccadic movements (97%), macular pigmentary changes (91%), ophthalmoplegia (64%), Hoffmann sign (48%), Babinski sign (45%), and ankle clonus (55%). The severity assessment of SCA7 was conducted using neurological scales SARA and INAS for the evaluation of cerebellar and noncerebellar symptoms, respectively (Table 1).

Table 1 depicts the determination of shimmer (amplitude disturbance), jitter (frequency disturbance), and fundamental frequency (number of vibrations per second of vocal folds, measured in Hertz) parameters;[13–15] interestingly, we recorded significantly higher shimmer and jitter values in patients with SCA7 than in control subjects ($P < 0.05$). Patients exhibited shimmer values that ranged from 1.50% to 39.09%, whereas control subjects displayed shimmer values that ranged from 1.85% to 6.55%. Likewise, scores for jitter ranged from 0.07% to 15.75% in patients with SCA7 compared with 0.09–1.32% in control subjects. Conversely, we obtained lower measures for fundamental frequency in patients with SCA7 (range, 94.1–239.15 Hz), compared with control individuals (range, 88.33–289.03 Hz) ($P < 0.05$).

Accordingly to age at onset of symptomatology, we had previously classified patients with early- and adult-onset disease.[3] Therefore, we analyzed whether acoustic voice parameters are related to disease onset. All of the three voice parameters, including jitter, shimmer, and fundamental frequency, showed similar scores between patients with early-onset disease and those with adult-onset disease, which implies that disease evolution does not modify voice quality (Figure 1).

Intriguingly, although the size of the CAG repeat tract influences disease severity, with longer expansions giving rise to earlier