Trinucleotide repeat analysis of spinocerebellar ataxia patients in Oman

Jacob P. Chacko, MD, DM, Shanmugakonar Muralitharan , MSc, M.Phil, Alia Al-Ansari, PhD, Khalsa Al-Kharusi, BSc, Abdullah Al-Asmi, FRCP, Chand R. Pratap, DM, FRCP, Riad Bayoumi, PhD, FRCP.

ABSTRACT

Objective: To explore the profile of cytosine/adenine/guanine (CAG) repeat expansion in Omani spinocerebellar ataxia (SCA) patients.

Methods: Ten SCA patients attending the Sultan Qaboos University Hospital Neurologic clinics, Al-Khoud, Oman in the 3 years starting from January 2000 were recruited for this study. Genomic DNA was extracted from peripheral blood samples and CAG repeat expansion analysis was carried out by polymerase chain reaction and sequencing, when required.

Results: The CAG triplet repeats leading to polyglutamine expansion and neurodegeneration are seen in spinocerebellar ataxias 1, 2, 3, 6, 7 and 17. By using primers for SCA 1, 2, 3 and 7, we found the repeats were in the normal range and triplet repeats do not seem to be a common cause for ataxia in Oman.

Conclusion: Spinocerebellar ataxia in Oman has the normal range of CAG repeats for the commonly found SCA1, SCA2, SCA3 and SCA7.

Neurosciences 2005; Vol. 10 (1): 61-63

Spinocerebellar ataxias (SCA) are diseases characterized by slow progressing limb and gait ataxia with other cerebellar signs, and sometimes with other neurological dysfunction. They are genetically determined, showing autosomal dominant inheritance, but may be sporadic. Since 1993, 15 genetic loci associations and 9 mutations have been found in this disease. The mutations have been repeats in different chromosomes, usually triplet repeats. Expanded cytosine/adenine/guanine (CAG) repeats, coding polyglutamine are found in SCA1, 2, 3, 6, 7, and 17.[1] Spinocerebellar ataxia type 3 is the most common, and with SCA1 accounts for more than 50% of SCA families. Earlier, we reported SCA patients with childhood onset and deafness.[2] We observed macular degeneration and epilepsy in some of our patients. These features are seen in SCA7 and hence we initiated this CAG expansion analysis of SCA1, 2, 3 and 7 in our subjects.

Methods. We studied 10 patients with progressive adult onset ataxia after excluding other causes by appropriate investigations, who were seen in the neurology clinic of Sultan Qaboos University Hospital, Muscat, Oman in the 3 years starting from January 2000. An MRI brain was carried out in all patients and showed no evidence of tumor or multiple sclerosis. Fresh EDTA blood samples were obtained after informed consent. Genomic DNA was isolated from peripheral blood leukocytes by standard procedures. The CAG repeat regions of SCA1, SCA2, SCA3 and SCA7 were amplified by polymerase chain reaction (PCR), in 50 microliter reaction volume using 10 picomoles of each primer,

From the Department of Neurology and Biochemistry (Chacko, Al-Kharusi, Al-Asmi, Pratap, Bayoumi), Sultan Qaboos University Hospital, and the Biology Department (Muralitharan, Al-Ansari), College of Science, Sultan Qaboos University, Al-Khoud, Muscat, *Sultanate of Oman*.

Received 28th June 2004. Accepted for publication in final form 18th August 2004.

Address correspondence and reprint request to: Dr. Shanmugakonar Muralitharan, Senior Biomedical Scientist, Department of Biochemistry, Sultan Qaboos University, PO Box 38, Al-Khoud 123, *Sultanate of Oman*. Tel. +968 513355. Fax. +968 515786. E-mail: smurli28 @hotmail.com

Table 1 - Primer sequences, polymerase chain reaction (PCR) conditions and calculation of the number of triplet repeats from the PCR products.

SCA	Forward Primer Sequence	Reverse Primer Sequence	Annealing temperature	Base pair/Repeat
SCA1	AACTGGAAATGTGGACGT	CAACATGGGCAGTCTGAG	54°C	215=30
SCA2	GGAGACCGAGGACGAGGA	CCTCACCATGTCGCTGAAG	56°C	184=20
SCA3	TGGCCTTTCACATGGATGTGA	CCAGTGACTTTGATTCG	55°C	226=22
SCA7	TTTTTTGTTACATTGTAGGAGCG	CACTTCAGGACTGGGCAGAG	58°C	291=10

SCA - spinocerebellar ataxia, A - adenine, C - cytosine, T - thymine, G - guanine

Table 2 - Pattern of CAG repeats in the study population.

SCA	Normal range of repeats	Affected range	Maximum in Oman
SCA1	19-38	40-82	38
SCA2	15-30	34-48	21
SCA3	14-30	68-83	23
SCA7	7-15	32-53	20

SCA - spinocerebellar ataxia

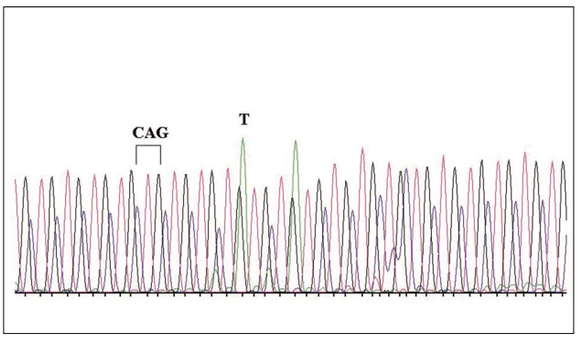

Figure 1 - DNA sequence showing the CAG repeats in SCA1 with 2 CAT interruptions. CAG - cytosine/adenine/guanine, T - thymine.

0.25mM of dNTP, one unit of amplitaq DNA polymerase and a buffer containing 10 mM Tris HCl, pH 8.3, 50 mM KCl and 0.01% gelatin. The details of the primers used are given in **Table 1**. Dimethyl sulphoxide, 10% was used for SCA2 and 7 amplification to prevent inter and intra strand annealing. Magnesium chloride concentration was 1.25 mM for SCA3, 1.5 mM for SCA1 and 2, and 3 mM for SCA7. The PCR products were analyzed in 2% agarose gel to which ethidium bromide was added. The amplified fragments were column purified, and cycle sequenced with the ABI PRISM Big Dye Termination ready reaction kit (PE Applied Biosystems) and analyzed by an ABI 3100 DNA sequencer. The details of the number of repeats are given in **Table 2**.

Results. The SCA was sporadic in 9 of our patients and familial in one in which, mother and 2 brothers were affected. There were 4 males and 6 females. The mean age of onset was 30 years, ranging from 8-50. All patients had gait and limb ataxia. Two had seizures, and one had myoclonic jerks. Five patients had slurring of speech and one was anarthric. Slow saccades were seen in 2 patients and saccades were absent in one. One patient had macular degeneration on ophthalmoscopic examination, although her vision was normal. There was no bulbar palsy, extrapyramidal features or neuropathy. Triplet repeat number was in the normal range in the case of SCA1, 2, 3 and 7 in all patients. The sequence data of SCA1 showed normal number of CAG repeats along with 2 cytosine/adenine/thymine (CAT) interruptions as is found in the normal population (**Figure 1**).

Discussion. The SCA1, 2, and 3 typically occur in young adults, SCA7 can have childhood onset and SCA 6, occurs in the elderly. The SCA 3, also known as Machado-Joseph disease, after the first patients in which it was diagnosed in the Portuguese Azores island, is the most common familial SCA. In addition to ataxia, they have extrapyramidal features, bulbar palsy and peripheral neuropathy. The abnormal gene with excess triplet repeats fails to degrade ubiquinated proteins in proteosomes. The SCA1 patients have bulbar palsy and sometimes respiratory difficulties and ophthalmoparesis in addition to the cerebellar ataxia. The misfolded, aggregated ataxin damages the nucleus of Purkinje

cells in the cerebellum. This occurs more frequently when the protein has a phosphorylated serine amino acid, some distance away from its expanded polyglutamine segment, in the presence of the protein 14-3-3 which acts as a stabilizer.[3] The SCA7 patients have macular degeneration, deafness and epilepsy and the abnormal protein is thought to have a role in acetylation of histone proteins, which are nucleoproteins. Though the latter clinical features were seen in our patients, they did not carry the SCA7 mutation

Commonly described SCA repeat expansion mutations seem to be uncommon in this region of the eastern part of the Arabian peninsula. Triplet and polymer repeats are usually described in large SCA families, and their positivity in mostly sporadic cases in our series of patients is low. In a recent Korean study,[4] 52 out of 253 cerebellar ataxia patients had triplet repeats in the pathogenetic range, involving SCA1, 2, 3, 6 and 7, and in a Japanese study,[5] expanded triplet repeats were found in 15% of sporadic cerebellar ataxia patients.

References

1. Paulson H. Polyglutamine degeneration: minding your Ps and Qs. ***Nat Med*** 2003: 825-826.
2. Pratap-Chand R, Gururaj AK, Dilip-Kumar S. A syndrome of olivopontocerebellar atrophy and deafness with onset in infancy. ***Acta Neurol Scand*** 1995; 91: 133-136.
3. Heintz N. Biomedicine: Ataxin 1 regulators in the spotlight. ***Science*** 2003; 301: 59-60.
4. Lee WY, Jin DK, Oh MR, Lee JE, Song SM, Lee EA, et al. Frequency analysis and clinical characteristics of spinocerebellar ataxia types 1, 2, 3, 6 and 7 in Korean patients. ***Arch Neurol*** 2003; 60: 858-863. Erratum in: ***Arch Neurol*** 2003; 60: 1256.
5. Matsumara R, Futumara N, Ando N, Ueno S. Frequency of spinocerebellar mutations in the Kinki district of Japan. ***Acta Neurol Scand*** 2003; 107: 38-41.