



## Negative results

Sequencing analysis of the spinal bulbar muscular atrophy CAG expansion reveals absence of repeat interruptions<sup>☆</sup>Pietro Fratta<sup>a,c,1,\*</sup>, Toby Collins<sup>a,1</sup>, Sally Pemble<sup>d</sup>, Suran Nethisinghe<sup>b</sup>, Anny Devoy<sup>a</sup>, Paola Giunti<sup>b,d</sup>, Mary G. Sweeney<sup>d</sup>, Michael G. Hanna<sup>c</sup>, Elizabeth M.C. Fisher<sup>a,c</sup><sup>a</sup> Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK<sup>b</sup> Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK<sup>c</sup> MRC Centre for Neuromuscular Disease, UCL Institute of Neurology, Queen Square, London, UK<sup>d</sup> Neurogenetics Unit, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK

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## ABSTRACT

Trinucleotide repeat disorders are a heterogeneous group of diseases caused by the expansion, beyond a pathogenic threshold, of unstable DNA tracts in different genes. Sequence interruptions in the repeats have been described in the majority of these disorders and may influence disease phenotype and heritability. Spinal bulbar muscular atrophy (SBMA) is a motor neuron disease caused by a CAG trinucleotide expansion in the androgen receptor (*AR*) gene. Diagnostic testing and previous research have relied on fragment analysis polymerase chain reaction to determine the *AR* CAG repeat size, and have therefore not been able to assess the presence of interruptions. We here report a sequencing study of the *AR* CAG repeat in a cohort of SBMA patients and control subjects in the United Kingdom. We found no repeat interruptions to be present, and we describe differences between sequencing and traditional sizing methods.

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## 1. Introduction

Spinal bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a slowly progressive, adult-onset, motor neuron disease, inherited in an X-linked recessive manner and caused by a CAG repeat expansion in the first exon of the androgen receptor gene (*AR*) (La Spada et al., 1991). Although normal individuals carry 9 to 36 CAG repeats, SBMA patients carry 38 to 62 repeats (La Spada et al., 1991). SBMA is part of a wider group of neurological diseases in which the causative genetic defect is a coding CAG expansion and for which the pathogenic threshold is approximately 35 to 40 repeats (Table 1) (Atsuta et al., 2006; Orr and Zoghbi, 2007). Interestingly, although most frequently the CAG expansions are pure runs of CAGs, sequence interruptions have been documented in the majority of these disease genes (Table 1).

Interruptions have been shown to play a role in determining the clinical phenotype and the disease heritability, and therefore carry potentially important information for genetic testing and counseling (Chong et al., 1995; Matsuyama et al., 1999; Yu et al., 2011). Pulic genomic databases report rare sequence variations (rs62636527, rs62636528, rs62636529) occurring with low frequency (0.013–0.043) in the *AR* CAG repeat, but SBMA diagnostic testing and previous studies have mostly used fragment sizing analysis to assess the presence and size of CAG repeats (Atsuta et al., 2006; La Spada et al., 1991), and have not been able to systematically address whether there are interruptions in SBMA expansions, thus warranting direct target sequence analysis.

## 2. Methods

Details of the patient cohorts, fragment sizing, and sequencing are given in the Supplementary text. The study was approved by the local ethical committee.

## 3. Results

To investigate the presence of interruptions in the *AR* CAG repeat, we sequenced the *AR* CAG repeat in a population of 40 SBMA patients and 93 control subjects in the United Kingdom. Overall, SBMA samples showed a median of 45 repeats (range, 40–53

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**Table 1**  
Summary of CAG characteristics in diseases caused by CAG coding expansions

Disease	Gene	No. of CAG repeats				Presence of interruptions in CAG repeat sequence	Reference
		Normal allele	Intermediate allele	Reduced penetrance allele	Fully penetrant allele		
DRPLA	ATN1	6–35	36–47		48–93	No	Koide et al., 1994
SCA1	ATXN1	6–38	36–38		39–91	Yes	Orr et al., 1993
SCA2	ATXN2	14–31			32–270	Yes	Pulst et al., 1996
SCA3	ATXN3	12–44		45–51	52–86	Yes	Kawaguchi et al., 1994
SCA17	TBP	25–40		41–48	49–66	Yes	Koide et al., 1999
HD	HTT	≤26	27–35	36–39	≥40	Yes	Huntington's Disease Collaborative Research Group, 1993
SBMA	AR	9–36	37		38–62	No	La Spada et al., 1991; present work

Key: SBMA, spinal bulbar muscular atrophy.

repeats; [Supplementary Fig. 1](#)), which is comparable to the results reported in previous U.S. and Japanese series ([Atsuta et al., 2006](#); [La Spada et al., 1991](#)). The quality of our direct sequencing of the CAG expansion allowed us to address the presence of interruptions by visual inspection in both forward and reverse sequences of the amplicon ([Supplementary Fig. 2](#)). We did not find interruptions in any of our SBMA case patients or control subjects ([Table 1](#)).

Interestingly, when comparing sizing obtained by sequencing to the fragment analysis used diagnostically, we observed a constant difference of 3 to 4 repeats, with estimation from fragment analysis being smaller. This was not dependent on the allele size, and was constant among pathological alleles and controls.

#### 4. Discussion

Diagnostic testing and previous large-scale studies have relied on polymerase chain reaction fragment analysis for sizing the CAG repeat in *AR*, and have therefore not been able to address the presence of sequence interruptions in a large number of samples ([Atsuta et al., 2006](#); [La Spada et al., 1991](#)). This is an important issue, because interruptions have been shown to be present in the majority of CAG expansion diseases and to play a role in clinical features of the disease, such as age of onset and presentation. In the case of ataxin 2 (*ATXN2*), the combination of presence of interruptions and repeat size determines very distinct clinical presentations that encompass SCA2, L-DOPA-responsive parkinsonism, and amyotrophic lateral sclerosis ([Matsuyama et al., 1999](#); [Yu et al., 2011](#)). Interruptions have also been shown to influence the capability of the repeat to expand in the germ line, therefore carrying potential implications for genetic counseling ([Chung et al., 1993](#)).

Importantly, our results show that interruptions are not frequent in the *AR* CAG repeat and are therefore not likely to play a role in disease, making SBMA an exception among coding CAG expansion diseases.

Our results also show that fragment analysis, used routinely for the diagnostic testing of the SBMA and other CAG expansion diseases, underestimates the CAG expansion. This discrepancy is present also when analysing CAG expansions from other genes ([Menon et al., 2013](#)). In diagnostic laboratories, this bias is corrected for by calibrating the fragment analysis with amplicons of known sizes. Our results indicate that direct sequencing of the *AR* CAG repeat polymerase chain reaction amplicon in males is a reliable method for sizing evaluation, and therefore we suggest that this may be useful when analyzing size lengths of the CAG repeat near the disease threshold of 38 repeats. This possibility is unique to SBMA testing in males and differs from all other triplet expansion diseases in which the presence of sequence variability and the effect of

having 2 alleles, makes direct polymerase chain reaction sequencing less informative.

#### Disclosure statement

The authors declare no actual of potential conflicts of interest.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.015>.

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