Analysis of spinocerebellar ataxias due to expanded triplet repeats in Greek patients with cerebellar ataxia

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1. Introduction

The autosomal dominant cerebellar ataxias (ADCA) are a group of clinically and genetically heterogeneous inherited disorders characterised by progressive cerebellar ataxia often associated with additional neurological features, such as pyramidal and extrapyramidal signs, ophthalmoplegia, cognitive impairment, peripheral neuropathy and optic atrophy [1]. They are referred to in the genetic literature as ‘spinocerebellar ataxias’ (SCAs) and are assigned ascending numerals chronologically [2]. At present over 35 genetically distinct SCAs have been identified [3].

The overall prevalence of ADCA in European populations is 1 to 3 per 100,000 [2]. The relative frequency of different SCAs varies significantly according to ethnic origin, partly due to founder effects [4]. The commonest SCAs in most populations are SCA1, SCA2, SCA3, SCA6 and SCA7, all caused by expanded triplet repeats. Triplet expansions also cause the less common SCA8, SCA12, SCA17 and DRPLA [2]. Although most patients with SCAs come from families exhibiting autosomal dominant (AD) inheritance, occasionally apparently sporadic cases can be attributed to a SCA mutation [5].

In the present study we sought to investigate the relative frequency of the triplet repeat expansion SCAs in a Greek population of familial and sporadic ataxia patients. To the best of our knowledge, no information exists to date regarding SCAs in Greece.

2. Methods

A total of 83 Greek patients exhibiting a slowly progressive cerebellar ataxia with or without additional neurological features were included in the study. They were consecutively referred to the Neurogenetics Unit of the Department of Neurology, University of Athens, Eginitio Hospital, for the exclusion of genetic causes of ataxia, over a period when only the Friedreich expansion was available diagnostically in our laboratory. Of the total cohort, 20 were probands from families exhibiting AD inheritance with affected members in successive generations. The total number of affected individuals still alive was 56. Another 7 patients belonged to families with more than one affected member in a single generation, suggestive of autosomal recessive inheritance, but also compatible in certain cases with dominant inheritance. Fifty six patients were isolated cases of ataxia, apparently sporadic. Demographic and clinical details of the patient

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cohort are shown in Table 1. Evaluation of alcohol consumption, brain MRI, CSF analysis, routine biochemical, haematological and immunological screens, thyroid function tests, as well as vitamins B12 and E were performed to exclude known acquired causes of progressive cerebellar ataxia. Additionally, all patients were tested and found negative for the triplet expansion causing Friedreich ataxia.

DNA was isolated from peripheral blood leucocytes. Written informed consent to perform molecular genetic studies was obtained from all patients and the study was approved by the ethics committee of Eginition Hospital. DNA was amplified by PCR using appropriate primer pairs for ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, ATXN8/ATXN8OSP, PP2R2B, TBP and ATN1 according to established protocols. PCR products were checked on a 4% agarose mini gel and then run on an ABI3730XL genetic analyzer with a LIZ500 size standard. Fragment analysis was performed with GeneMapper version 3.7 software. Statistical analysis was performed on SPSS version 13.0.

3. Results
In total, 4 patients with pathological SCA expansions were found. They all belonged to the group with clear AD inheritance, comprising 20% of these patients (Table 1). Two patients had SCA1 (10.0% of AD group), one SCA2 (5.0% of AD group) and one SCA7 (5.0% of AD group). The remaining patients with AD disease (80%) were negative for expansions in the loci tested. No pathological expansions were found in the group with probable recessive or unclear inheritance pattern. Likewise, no abnormal expansions were detected in the group with apparently sporadic ataxia. Overall, no patients with SCA3, SCA6, SCA12, SCA17 or DRPLA were identified.

Clinical features of patients with pathological expansions are shown in the Supplementary table. No patient had a pure cerebellar ataxia. Both SCA1 patients exhibited pyramidal signs in addition to ataxia, the patient with longer disease duration also exhibiting ophthalmoplegia, sensory signs and mild cognitive impairment. The SCA2 patient had slow saccades and hyporeflexia. The patient with the SCA7 expansion had additional retinopathy, expressed clinically as a dyschromatopsia, optic atrophy and pyramidal signs.

4. Discussion
The present study provides information regarding the frequency of SCAs in the Greek population. To the best of our knowledge, there have been no previous reports to date on this subject. Given results from previously studied populations, we were expecting to find a genetic cause in at least 50% of our AD ataxia cases [2]. However, only 20% of this group proved to carry one of the tested expansions. A similarly high proportion of unexplained AD ataxia pedigrees has been reported from Norway and the UK [11,12]. It remains to be determined what the underlying genetic cause in these patients is. Possible founder effects involving any of the known SCAs that were not tested or other unknown mutations could partly explain the present finding.

Table 1
Demographic, clinical and genetic characteristics of the patient cohort.

|                  | Autosomal dominant | Other familial | Sporadic | Total |
|------------------|--------------------|---------------|----------|-------|
| N                | 20                 | 7             | 56       | 83    |
| Sex (F/M)        | 11/9               | 3/4           | 30/26    | 44/39 |
| Age (yrs)        | 44.1 ± 12.7        | 43.9 ± 10.0   | 41.4     | 42.2  |
| Age at onset (yrs) | 30.3 ± 13.0      | 19.7 ± 18.0   | 27.9     | 27.7  ± 20.5 ± 18.7 |
| Early/late onset*| 5/15               | 2/5           | 27/29    | 34/40 |
| SCA1 (%)         | 2 (10.0)           | 0             | 0        | 2 (2.4) |
| SCA2 (%)         | 1 (5.0)            | 0             | 0        | 1 (1.2) |
| SCA7 (%)         | 1 (5.0)            | 0             | 0        | 1 (1.2) |
| SCA3, SCA6, SCA8, SCA12, SCA17, DRPLA | 0 | 0 | 0 | 0 |
| Total SCA positive (%) | 4 (20.0) | 0 | 0 | 4 (4.8) |

* Early onset defined as <25 years; SCA: spinocerebellar ataxia; DRPLA: dentatorubral-pallidoluysian atrophy.

No SCA triplet expansions were detected in any of the patients from the probably recessive/unclear inheritance group or the sporadic group. In the familial group this would be expected if these patients indeed represented recessive pedigrees. In the case of sporadic ataxias, previous studies have reported somewhat varying results, with SCA expansions found in less than 1% of patients in some series [9,12] and up to 10% in other series [5,13]. Interestingly, SCA6 is the most common SCA expansion detected in these patients (6–7%). If therefore the underlying prevalence of SCA6 is very low in the population under study, this would be expected to influence the number of sporadic cases carrying an expansion. This was the case in the Spanish population and may also influence the findings in Greek patients [9]. Another factor possibly contributing to the absence of SCA expansions in the sporadic cases was the significant proportion of early onset cases, possibly reflecting an unidentified underlying recessive cause [14]. Of course, the strictness of inclusion criteria in sporadic cases could also substantially influence the results in this group of patients.

In conclusion, the present study provides the first data on the frequency of SCAs due to triplet expansions in the Greek population. Patients with SCA1, SCA2 and SCA7 were identified, but SCA3 and SCA6 were not found. The genetic cause for the majority of AD pedigrees remains to be identified. In sporadic cases no SCA expansions were found.

Supplementary data to this article can be found online at doi:10.1016/j.jns.2012.03.019.

Conflict of interest
The authors report no conflict of interest.

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