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Friedreich's ataxia and other hereditary ataxias in Greece: An 18-year perspective

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ABSTRACT

Limited data exist on the spectrum of heredoataxias in Greece, including the prevalence and phenotype of Friedreich's ataxia (FRDA) and the prevalence and subtypes of dominant spinocerebellar ataxias (SCAs). We analyzed clinically and investigated genetically for FRDA and triplet-repeat expansion SCAs a consecutive series of 186 patients with suspected heredoataxia referred to Athens over 18 years. For prevalence estimates we included patients with molecular diagnosis from Cyprus that were absent from the Athens cohort. The minimum prevalence of FRDA was ~0.9/100,000, with clusters of high prevalence in Aegean islands. FRDA was diagnosed in 73 probands. The genotypic and phenotypic spectrum of FRDA was similar to other populations, with one patient compound heterozygote for a known point mutation in *FXN* (Asn146Lys). Undiagnosed recessive ataxias included FRDA-like and spastic ataxias. The minimum prevalence of dominant SCAs was ~0.7/100,000. SCA1 (4), SCA7 (4), SCA2, SCA6, and SCA17 (1 each) probands were identified. A molecular diagnosis was reached in 31% of dominant cases. Undiagnosed dominant patients included a majority of type III autosomal dominant cerebellar ataxias. FRDA is the commonest heredoataxia in the Greek population with prevalence towards the lower end of other European populations. Dominant SCAs are almost as prevalent. SCA1, SCA2, SCA6, SCA7 and SCA17 patients complete the spectrum of cases with a specific molecular diagnosis.

1. Introduction

Hereditary ataxias are a group of heterogeneous genetic disorders causing degeneration of the cerebellum and its connections and resulting in gait unsteadiness, limb incoordination, dysarthria and oculomotor dysfunction. They often have additional neurological features partly depending on the underlying genetic subtype. In the majority of cases they are inherited in an autosomal recessive or autosomal dominant manner [1].

Friedreich's ataxia (FRDA) is the commonest recessive ataxia in Caucasians with a prevalence of ~2/100,000 [2]. It is thought to constitute up to 75% of recessive ataxias in Europeans [2]. The development of precise clinical criteria for FRDA paved the way for numerous epidemiologic studies in different European populations [3]. The subsequent cloning of the gene allowed broadening of the phenotype and inclusion of atypical forms [4,5]. To date, there is no information on the prevalence and phenotypic spectrum of FRDA in Greece. Furthermore, there are no data on the relative frequency of FRDA within the broader ataxia spectrum in the Greek population.

Dominant ataxias, known as spinocerebellar ataxias (SCAs), have a prevalence of ~1–3/100,000 in Europeans, although this can vary significantly among different populations [6]. They can be divided phenotypically into autosomal dominant cerebellar ataxia (ADCA) types I, II and III based on the presence of extra-cerebellar findings [7]. We have recently published data on the relative contribution of triplet-repeat expansion SCAs in the Greek ataxia population [8]. Updated information on dominant ataxias has been incorporated into this study to complete the spectrum of familial ataxias in Greece.

In the present study spanning an 18-year period we provide comprehensive data on hereditary ataxias in the Greek population calculating a minimum prevalence for FRDA and SCAs and placing them within the broader spectrum of hereditary ataxias.

2. Methods

2.1. Patients

The Neurogenetics Unit at the 1st Department of Neurology, University of Athens Medical School, Eginition Hospital is the only unit

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of its kind in Greece and has acted as referral center for patients with presumed inherited ataxias since 1995. It has been the only laboratory within the public health service offering molecular diagnostic testing for FRDA to patients from all over Greece. Although acting as a referral center for all inherited ataxias, including dominant families, collecting clinical data and storing DNA, it has only recently offered diagnostic testing for SCA1, 2, 3, 6 and 7. The Cyprus Institute of Neurology and Genetics has acted as a referral center for patients from Cyprus with neurogenetic disorders since 1990. Occasionally, it also provides molecular diagnosis to Greek patients resident in Greece.

The present study includes a cohort of 186 consecutive index patients of Greek origin with suspected inherited ataxia referred to the Athens Neurogenetics Unit from all regions of Greece from 1995 to 2012. Of these, 131 were examined by neurologists of the Unit (96 in the specialist neurogenetics clinic and 35 as in-patients at Eginition Hospital) and 55 were seen by other neurology or pediatric departments from all over the country and their DNA samples and clinical notes were sent to the Unit for assessment and molecular diagnostic testing. Non-index cases still alive were 11 for FRDA, 42 for dominant ataxias and 18 for other recessive ataxias. We compared the Athens ataxia cohort to ataxia patients of Greek origin resident in Greece referred to the Cyprus Institute for molecular diagnosis and indentified 9 probands and 4 non-index cases homozygous for the FRDA expansion, one patient with a SCA1 expansion and one with a SCA6 expansion that were not present in the Athens cohort. For minimum prevalence estimations we have included these patients. However, all other clinical and genetic analyses have been restricted to the Athens cohort.

2.2. Demographic data

The minimum prevalence of FRDA and SCAs in the Greek population was estimated based on the census of 2001. The total population of Greece was 10,934,067 including 761,813 foreigners, resulting in 10,172,284 individuals of Greek origin [9].

2.3. Molecular genetic analysis

Patients gave informed consent for the performance of molecular diagnostic testing and the study was approved by the Eginition Hospital ethics committee. DNA was isolated from peripheral blood leucocytes. All patients were tested for the FRDA expansion in *FXN* according to established protocols [10]. PCR products were checked on 2% agarose gels. Two patients heterozygous for the FRDA expansion were further analyzed with sequencing of the 5 coding exons of *FXN* [4]. Eighty three FRDA negative patients have been included in a recently published study and tested in London for mutations causing SCA1, 2, 3, 6, 7, 8, 12, 17 and DRPLA [8]. The remaining FRDA negative patients were tested in Athens for SCA1, 2, 3, 6, 7, 17 and DRPLA according to established protocols. PCR products were checked on 4% agarose gels and then run on an ABI310 genetic analyzer with a TAMRA 500 size standard. Fragment analysis was performed with GeneScan version 3.7 software. Statistical analysis was performed on SPSS version 16.0 using chi-square and Fischer's exact test as appropriate.

3. Results

3.1. Minimum prevalence of Friedreich's ataxia and clusters of high prevalence

In total, 88 patients of Greek origin still alive with FRDA were identified. This gives an overall minimum prevalence of 0.87/100,000 for FRDA in the Greek population. Cases were in general uniformly distributed across different geographical regions of Greece with the exception of the Aegean islands, which had an increased prevalence of 2.2/100,000 (p 0.002). More specifically, clusters of FRDA cases were identified in the Aegean islands of Ikaria (5 cases in 12,279, p 0.000; prevalence 40.7/100,000) and Melos (2 cases in 5129, p 0.001; prevalence 39.0/100,000). One family from Ikaria exhibited 'pseudodominant' inheritance and has been previously published [11]. The number of molecular diagnoses of FRDA per year in the Athens Neurogenetics Unit was higher in the first 4 years following introduction of genetic testing and then gradually decreased, stabilizing around 2005 at 2–3 new cases per year, suggesting that we have reached a steady state.

3.2. Minimum prevalence of dominant spinocerebellar ataxias (SCAs)

Including non-index cases, 70 patients with dominant ataxia still alive were identified (excluding one sporadic SCA7 patient). This gives an overall minimum prevalence of 0.68/100,000 for SCAs in the Greek population.

3.3. Classification of ataxias

Ataxia index patients referred to the Athens Neurogenetics Unit were divided into familial and sporadic. Familial cases were further subdivided into dominant and suspected recessive and sporadic cases into early and late-onset (late onset defined as over 25). The above are shown as a tree diagram in Fig. 1. The diagram and Table 1 display the number of cases with a final molecular diagnosis of FRDA (excluding the patient with a single expanded FRDA allele but no identified point mutation) or one of the SCAs. Table 1 also displays basic demographic, clinical and genetic characteristics of the entire patient cohort, comparing familial and sporadic cases. FRDA was the molecular diagnosis in 34.4% of all index patients. A known SCA mutation was found in 4.8% of patients. Sporadic cases had significantly more patients with early onset and significantly more patients with FRDA than familial cases. In total, 42% of recessive and 40% of sporadic (54% of early and 11% of late-onset) ataxia patients had molecularly confirmed FRDA (Fig. 1). Grouping together recessive and early-onset sporadic cases (both likely to harbor recessively inherited mutations) gave a 51.3% for FRDA. Excluding dominant ataxia, FRDA represented 40.0% of all ataxia index cases in the Athens cohort.

3.4. Clinical and genetic characteristics of patients with Friedreich's ataxia

Table 2 summarizes the clinical and genetic characteristics of index patients with FRDA referred to the Athens Neurogenetics Unit. Over 90% of patients had the classic FRDA phenotype [3]. The frequency of typical clinical features of FRDA in these patients is shown in Table 2. In total, 9.4% of index patients had late onset (>25 yrs). Additionally, one sibling of an index patient had very late onset (>40 yrs). Six patients (9.4%), all with late onset, had retained lower limb reflexes (5 increased, 1 present). Age at onset of FRDA with retained lower limb reflexes was significantly different from classic FRDA (32.5 ± 4.8 vs. 14.0 ± 5.3, $p = 0.000$). Lower and upper GAA repeat number was significantly less in index patients with retained reflexes (GAA1: 316.3 ± 182.3 vs. 697.0 ± 194.8, $p = 0.000$; GAA2: 613.3 ± 259.4 vs. 960.7 ± 280.8, $p = 0.003$).

Out of two sporadic patients with clinical features suggestive of FRDA and a single expanded allele, we detected a known point mutation in one patient. Thus, compound heterozygotes comprised 1.6% of FRDA index cases in the Athens cohort. The patient had a c.438 C>G (p. Asn146Lys) point mutation and a single expanded allele of 702 GAA repeats. She had a typical FRDA phenotype with onset at 11 years, ataxia, dysarthria, no nystagmus, absent reflexes, Babinski signs, pes cavus and a brain MRI without significant cerebellar atrophy. Disease duration was approximately two years and there were no follow-up data.