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High frequency of Friedreich's ataxia carriers in the Paphos district of Cyprus

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A cluster of Friedreich's ataxia patients has been previously investigated in two neighbouring villages of the Paphos district of Cyprus. Molecular genetic studies revealed that all patients had the most common mutation, a homozygous expansion of the GAA triplet repeat in the first intron of the frataxin gene. The present study is aimed at estimating the mutation carrier frequency in the broader area of Paphos. Overall, 1050 individuals originating from the Paphos district took part in the programme. Blood samples were collected for a period of 18 months, on a voluntary basis, after signing a consent form, and analysis of the GAA triplet repeat was performed. The frequency of mutation carriers in the broader area of the Paphos district, and excluding the two neighbouring cluster villages, is estimated to be high. We recommend that an organized prevention programme be implemented to cover the population from this region.

Key words: Friedreich's ataxia, GAA repeat expansion, population screening, high carrier frequency

Introduction

Friedreich's ataxia (FRDA) is the most common of the hereditary ataxias. It is an autosomal recessive neurodegenerative disease (1, 2), has a prevalence of approximately 2 x 10^{-5} in Caucasian populations and the carrier frequency is estimated to be 1 in 90. Local clusters due to a founder effect have been reported in Rimouski, Quebec (3) and Paphos, Cyprus (4).

The majority of FRDA patients are homozygous for an unstable GAA trinucleotide repeat expansion in the first intron of the frataxin (FXN) gene on chromosome 9q13. Normal chromosomes have 8-33 GAA repeats while FRDA chromosomes have 67-1300 GAA repeats. Detection of the expansion mutation provides a very useful diagnostic test.

In 1988, Dean et al. (4) reported on the evaluation of 13 FRDA patients belonging to 7 Cypriot families originating from the neighbouring villages of Kathikas and Arodhes in the Paphos district of Cyprus. They concluded that the FRDA mutation frequency in these two villages must be the highest recorded, and was estimated to be 1-in-6 to 1-in-7 of the population. Since this initial report, 13 additional Cypriot FRDA patients have been observed; 11 of them originating from Paphos (incidence of ~ 1 per year for a population of ~50,000) and 10 out of the 11 with no evidence of Kathikas-Arodhes origin. This observation prompted us to investigate the existence of FRDA mutation carriers in the general population of the Paphos district in an attempt to map the spread of the mutation outside the villages of Kathikas and Arodhes. All Cypriot FRDA patients have been diagnosed at the molecular genetic level and proved to be homozygous for the GAA repeat expansion mutation.

Materials and methods

We initiated a screening programme in the population originating from the Paphos district which was carried out for 18 months. The aims of the programme were: 1) to inform the population about the disease, the mode of inheritance and available diagnostic options; 2) to collect samples from individuals, after informed and signed consent, in an attempt to better estimate the FRDA mutation frequency in the greater area of the Paphos district, and 3) to offer further genetic counselling to the FRDA mutation carriers.

A leaflet with the relevant facts about FRDA and its high prevalence in the region was prepared and distributed to residents of the Paphos district through the local hospitals and local newspapers. Many field trips were carried out for organized talks in the city of Paphos and various village centres where collection of blood samples was also offered to participants after the talk. Genetic counselling sessions for carriers were organized at local hospitals. The study was approved by the Ethics Committee of the Cyprus Institute of Neurology and Genetics. Participation was voluntary and blood was collected after a consent form was signed. Overall, 1050 individuals aged >18 years took part in the programme.

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DNA was extracted from blood samples using standard salting out procedures (5). Each DNA sample was analyzed twice in a different experiment by polymerase chain reaction (PCR) amplification and agarose gel electrophoresis following the procedure described by Filla et al. (6).

Results

A total of 1050 individuals originating from the Paphos district were analyzed. The number of carriers identified in three different residential and in two origin classes is shown in Table 1. In order to estimate the carrier frequency of the mutation in the broader area of the Paphos district, the 146 participants that have an origin from Paphos but do not live in the Paphos district and the 46 residents of the cluster villages (Kathikas-Arodhes) were excluded; therefore, 858 individuals were considered. Of these, 78 were found to be carriers of the mutation, accounting for 9.09% or 1 in 11 individuals. Paternal and maternal origin was requested during the sampling process, and individuals reported the villages from where their parents originated. Interestingly, among the 98 carriers, many reported both parents originating from villages other than the two cluster villages. Molecularly identified carriers originate from a number of other villages as shown in Figure 1.

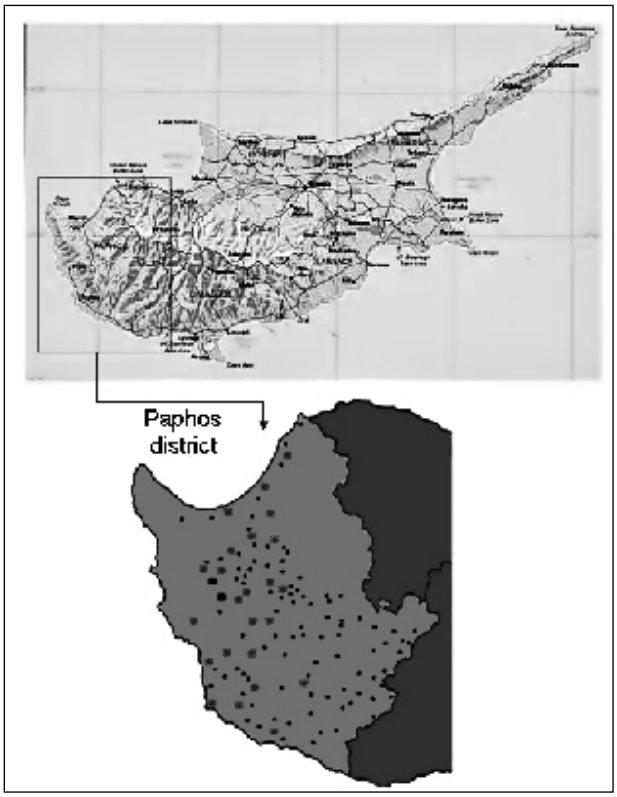

Figure 1. Map of Paphos, Cyprus. The cluster of patients was initially identified in the villages indicated with black squares. Using this programme, carriers of the FRDA mutation were identified in individuals originating from the villages/cities indicated with grey squares._The remaining villages of the Paphos district are indicated with black circles.

Discussion

The programme has been successful because the population has been informed about the high prevalence of FRDA in the region. Carriers that were diagnosed through this programme have been offered genetic counselling and they are aware of the risks and the available options. Many members of the previously ascertained FRDA families were already tested for their carrier status through our molecular diagnostic laboratory and did not take part in this study. Despite this observation, the risk of bias, due to participation of individuals with a positive family history, cannot be completely excluded. However, it is evident through this programme that there is a wider spread of the mutation beyond the Kathikas-Arodhes nucleus and that the carrier frequency is much higher than the reported 1 in 90 in Caucasian populations. In our opinion, the result of this programme urges the implementation of an FRDA prevention programme to cover the population originating from Paphos. The authorities of Cyprus have been informed and further continuation will depend upon the decision of the Cyprus Ministry of Health.

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Table 1. Carrier status of participants classified according to region of residence. All participants originate from the Paphos district.

Residence	Carriers (%)	Non-carriers (%)	Total
Kathikas-Arodhes	5 (10.87)	41 (89.13)	46
Paphos elsewhere	78 (9.09)	780 (90.91)	858
Other districts of Cyprus	15 (10.27)	131 (89.73)	146
	98 (9.33)	952 (90.67)	1050

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