MOLECULAR ANALYSIS OF FRIEDREICH'S ATAXIA IN MACEDONIAN PATIENTS

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ABSTRACT

Friedreich's ataxia (FRDA) is rare a progressive neurodegenerative disorder of autosomal recessive inheritance, which is associated with an unstable expansion of a GAA trinucleotide repeat in the first intron of the frataxin gene on chromosome 9q13. We have performed molecular analyses of the frataxin gene of 40 patients with spinocerebellar ataxia from the Republic of Macedonia. Fifteen had early onset of progressive ataxia (before the age of 25), while the remainder were over 25 years old at the time of diagnosis. Only 14 patients had a mutation in the frataxin gene and all of these had early onset ataxia. The number of GAA repeats was in the normal range in 50 healthy individuals.

INTRODUCTION

Friedreich ataxia (FRDA), is a progressive neurodegenerative disorder of autosomal recessive inheritance [1]. It is the most common hereditary ataxia, with an estimated prevalence of 1 in 50,000 [2-4], and a carrier frequency of about 1 in 120 in

the Caucasian population [5]. The cardinal feature is gait ataxia followed by upper limb ataxia, cerebellar dysarthria, nystagmus, areflexia, loss of joint position sense and spastic paraparesis [6], developing from the second decade of life. Hypertrophic cardiomyopathy occurs in almost all patients [7], diabetes mellitus in about 10% of patients, carbohydrate intolerance in an additional 20%, and a reduced insulin response to arginine stimulation in all patients [8].

Friedreich's ataxia is associated with an unstable expansion of a GAA trinucleotide repeat in the first intron of the frataxin gene on chromosome 9q13 [9]. Normal alleles contain five to 60 GAA repeats. The number of repeats in FRDA patients varies from 66 to 1,700, and results in a decreased expression of this gene [9-12], which probably leads to mitochondrial iron accumulation and free radical damage of oxidative phosphorylation processes [13,14]. In about 96% of the patients, both alleles are expanded, while 4% of patients are compound heterozygotes for GAA expansion in the disease-causing range and an inactivating mutation [9].

Friedreich's ataxia shows broad clinical variability [15,16]. The Harding diagnostic criteria can be used to differentiate between typical and non typical FRDA cases, but the differential diagnosis from some of the juvenile spinocerebellar ataxias remains a clinical problem. Molecular analysis can be useful to confirm clinical diagnosis and to detect carriers.

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MATERIALS AND METHOD

Patients and Controls. Forty patients from the Republic of Macedonia with spinocerebellar ataxia referred to our institution for molecular diagnosis of FRDA, were studied. Fifteen had an early onset of progressive ataxia (before 25 years), while 25 were older than 25 years at the time of diagnosis. Fifty healthy individuals were also studied.

GAA Repeat Analysis. Genomic DNA was extracted from venous blood leukocytes using a standard phenol/ chloroform extraction procedure [17]. Amplification of normal and expanded alleles was obtained by long range polymerase chain reaction (PCR) procedures using primers that flank the GAA repeat in intron 1 of the frataxin gene (GAAT: 5'-GGG ATT GGT TGC CAG TGC TTA AAA GTT AG-3' and GAAR: 5'-GAT CTA AGG ACC ATC ATG GCC ACA CTT GCC-3') and generate a PCR product of 457 + 3n bp (n = number of GAA repeats). Long-range PCR conditions, which gave efficient amplification, consisted of: 20 cycles at 94°C's for 20 seconds, 68°C for 2 min. and 72°C for 2 min., followed by 17 cycles in which the length of the 68°C steps was increased by 15 seconds per cycle. The PCR products were analyzed by 1% agarose gel electrophoresis, visualized by ethidium bromide

staining and recorded on a digital camera. The size of the PCR products was determined by comparing their migration rate with a molecular weight size standard [DNA molecular weight marker IX, 72-1353 bp (GE Healthcare Bio-Sciences AB, Uppsala, Sweden)].

RESULTS AND DISCUSSION

Representative electrophoregrams of PCR fragments from the amplification of GAA repeats in intron 1 of the frataxin gene in patients with FRDA and in healthy individuals are shown in Figure 1A and 1B, respectively. Polymerase chain reaction analyses of heterozygotes for expanded GAA alleles are shown in Figure 2.

Of the 15 patients diagnosed with spinal cerebellar ataxia before 25 years of age, 14 (93.3%) were homozygous for the GAA expansion and all had two expanded alleles of same size and no detectable somatic instability. Only one patient with early onset of spinal cerebellar ataxia had two normal alleles, and this result excluded the diagnosis of FRDA. The 25 patients with late onset spinal cerebellar ataxia had two alleles in the normal size range (5-50 GAA repeats). Ten of these were heterozygotes and 15 were homozygous for normal alleles

Figure 1. Agarose gel electrophoresis of PCR products of intron 1 of the frataxin gene in healthy individuals (lanes 1-7) with different numbers of GAA repeats (A), and of seven patients with FRDA (lanes 1-7) (B); M: molecular weight marker.

Figure 2. Agarose gel electrophoresis of PCR products of patients (lanes 2, 5 and 6) and members of families with FRDA (lanes 1 and 3), showing the normal and expanded fragment (lanes 1 and 3); M: molecular weight marker.

which excluded the diagnosis of FRDA. Molecular analysis of intron 1 of the frataxin gene in healthy individuals showed a range of GAA repeats from 5 to 50. Our results are concordant with already published data [1-4]. This long-range PCR protocol can be used as a diagnostic tool for FRDA and carrier detection.

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