Pathologie Biologie 62 (2014) 41–48

Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original article

A mitochondrial implication in a Tunisian patient with Friedreich's ataxia-like

Une implication mitochondriale chez un patient tunisien atteint d'une ataxie de Friedreich-like

M. Maalej [a], E. Mkaouar-Rebai [a,*], M. Mnif [b], N. Mezghani [a], I. Ben Ayed [a], I. Chamkha [a], M. Abid [b], F. Fakhfakh [a]

[a] *Laboratoire de génétique moléculaire humaine, faculté de médecine de Sfax, avenue Magida Boulila, 3029 Sfax, Tunisia*
[b] *Service d'endocrinologie, CHU Hédi Chaker de Sfax, avenue Magida Boulila, 3029 Sfax, Tunisia*

ARTICLE INFO

Article history:
Received 13 April 2013
Accepted 5 July 2013

Keywords:
Friedreich's ataxia
FXN
Twinkle
POLG1
MT-DLoop
mtDNA

ABSTRACT

Genes encoding the DNA helicase TWINKLE (*C10orf2*) or the two subunits of mtDNA polymerase γ (POLγ) (*POLG1* and *POLG2*) have a direct effect on the mitochondrial DNA replication machinery and were reported in many mitochondrial disorders. Friedreich's ataxia (FRDA) is the common cause of ataxia often associated with the expansion of a GAA repeat in intron 1 of the frataxin gene (*FXN*). Mitochondrial DNA could be considered as a candidate modifier factor for FRDA disease, since mitochondrial oxidative stress is thought to be involved in the pathogenesis of this disease. We screened the *FXN*, *POLG1* and *C10orf2* genes in a Tunisian patient with clinical features of Friedreich's ataxia-like. The results showed the absence of the expansion of a GAA triplet repeat in intron 1 of the *FXN* gene. Besides, the sequencing of all the exons and their flanking regions of the *FXN*, *POLG1* and *C10orf2* genes revealed the presence of intronic polymorphisms. In addition, screening of the mtDNA revealed the presence of several mitochondrial known variations and the absence of mitochondrial deletions in this patient. The detected m.16187C>T and the m.16189T>C change the order of the homopolymeric tract of cytosines between 16184 and 16193 in the mitochondrial D-loop and could lead to a mitochondrial dysfunction by inhibiting replication and affecting protein involved in the replication process of the mtDNA which could be responsible for the clinical features of Friedreich ataxia observed in the studied patient.

Mots clés:
Ataxie de Friedreich
FXN
C10orf2
POLG1
D-Loop
ADNmt

RÉSUMÉ

Les gènes codant pour l'ADN hélicase TWINKLE (C10orf2) ou les deux sous-unités de la polymérase γ de l'ADNmt (POLγ) (POLG1 et POLG2) ont un effet direct sur la machinerie de réplication de l'ADN mitochondrial et ont été associés à de nombreuses maladies mitochondriales. L'ataxie de Friedreich (FRDA) est la cause la plus fréquente de l'ataxie souvent associée à l'expansion d'une répétition GAA au niveau de l'intron 1 du gène de la frataxine (*FXN*). L'ADN mitochondrial pourrait être considéré comme un facteur modificateur de l'ataxie de Friedreich, puisque le stress oxydatif mitochondrial est impliqué dans la pathogenèse de cette maladie. Nous avons étudié les gènes *FXN*, *POLG1* et *C10orf2* chez un patient Tunisien présentant les signes cliniques de l'ataxie de Friedreich-like. Les résultats retrouvés ont montré l'absence de l'expansion du triplet GAA au niveau de l'intron 1 du gène *FXN*. Par ailleurs, le séquençage de tous les exons et les régions flanquantes des gènes *FXN*, *POLG1* et *C10orf2* a révélé la présence de polymorphismes introniques. En outre, l'étude de l'ADN mitochondrial a révélé la présence de plusieurs variations mitochondriales connues mais l'absence de délétions mitochondriales chez ce patient. Les variations m.16187C>T et m.16189T> C les cytosines entre les nucléoties 16184 et 16193 au niveau de la D-loop mitochondriale et pourrait conduire à un dysfonctionnement mitochondrial par inhibition des protéines impliquées dans le processus de réplication de l'ADN mitochondrial, ce qui pourrait être responsable de l'atteinte observée chez le patient étudié.

* Corresponding author.
E-mail address: emna_mkaouar@mail2world.com (E. Mkaouar-Rebai).

0369-8114/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.patbio.2013.07.013

1. Introduction

The etiology of mitochondrial disease is complex and can include abnormalities in respiratory chain function due to dysfunction in structural or assembly proteins, translation and maintenance of the mitochondrial DNA (mtDNA) [1]. Indeed, mutations in an increasing number of nuclear genes involved in the maintenance of mtDNA are being described, associated with an extensive spectrum of clinical phenotypes ranging from severe encephalopathy in infancy and childhood to late-onset progressive external ophthalmoplegia (PEO), ataxia, and myopathy. These mutations are located essentially in genes encoding the DNA helicase TWINKLE (*PEO1* or *C10orf2*) [2] or the two subunits of mtDNA polymerase γ (POLγ) (*POLG1* and *POLG2*) [3] which have a direct effect on the mtDNA replication machinery.

C10orf2 gene codes for the mitochondrial helicase Twinkle, which is one of the important proteins for mtDNA replication and maintenance working in close connection with mtDNA polymerase gamma POLG and have a direct effect on the mtDNA replication machinery [4].

Human mitochondrial DNA polymerase gamma is responsible for replication and repair of the mitochondrial genome [5]. Variations in the *POLG1* gene, which encodes the catalytic subunit of the mtDNA are associated with an expanding phenotypic spectrum ranging from severe encephalopathy and liver failure in childhood to late-onset external ophthalmoplegia, neuropathy, dysarthria, myopathy and ataxia [6,7].

Recessive *C10orf2* mutations were reported in hepatocerebral mtDNA depletion disorder and infantile-onset spinocerebellar ataxia (IOSCA) which is a severe autosomal recessively inherited neurodegenerative disorder [8–10]. It manifests at the age of 9 to 18 months and includes ataxia, athetosis, muscle hypotonia and loss of deep tendon reflexes. This ataxia is close to Friedreich's ataxia (FRDA) which is the most common recessively inherited ataxia [11], with an estimated prevalence of 1 out of 50,000 in the Caucasian population [12].

The disease onset is usually within the second decade of life and the major features include progressive ataxia of limbs and gait, dysarthria, loss of tendon refexes, pes cavus, scoliosis, pyramidal weakness, and predominantly sensory axonal neuropathy in association with hypertrophic cardiomyopathy and increased incidence of diabetes [13].

FRDA is known to be caused by the lack of frataxin, a nuclear DNA-encoded mitochondrial protein of 210 aminoacids located within mitochondrial inner membrane and crests and encoded by the *FXN* gene (or *X25* gene) on chromosome 9q13 [13]. Most patients with FRDA present the expanded GAA triplet-repeat sequences in intron 1 of the *FXN* gene leading to the formation of a "sticky" triplex DNA structure that interferes with correct transcription and reduces the synthesis of frataxin [14]. In *FXN* gene, normal alleles contain between seven and 38 GAA repeats, while pathologic alleles typically have between 66 and 1500 repeats [15,16]. The length of the shorter GAA repeat correlates with age of onset in patients with FRDA [14,17,18]. Approximately 95% of patients are homozygous for a GAA expansion; the remaining patients are compound heterozygous with an expanded GAA repeat on one allele and a point mutation on the other allele [19]. Besides, several mutations were reported in the *FXN* gene in patients with FRDA [20–23].

In the present study, we reported a mutational analysis of the mtDNA and the nuclear *FXN*, *C10orf2* and *POLG1* genes in a Tunisian patient with clinical features of Friedreich's ataxia-like including cerebellar and pyramidal syndrome, ataxia, scoliosis, pes cavus, claw toes, lumbar lordosis, dysmorphic syndrome, and diabetes.

2. Patient and methods

2.1. Patient

The patient was the third child born in 1980 of consanguineous Tunisian parents. He had a brother and two maternal uncles who died from ataxia (Fig. 1). He was diagnosed for FRDA since the age of 13-year-old.

Neurological examination showed a cerebellar and pyramidal syndrome and the electromyogram analysis showed the presence of a vestibular syndrome. He also suffers from ataxia, scoliosis, foot deformity (pes cavus), claw toes and lumbar lordosis.

Laboratory explorations revealed an elevated glucosuria and acetonuria and a high glycemia level (21 nmol/L; 4.75 g/L). His diabetes was detected at the age of 29-year-old. Thus, these clinical and neurological features were highly suggestive of a Friedreich' ataxia.

In addition, 200 Tunisian healthy individuals from the same ethnocultural group were tested as controls. These controls had no personal or family history of any disorder.

2.2. Methods

2.2.1. DNA extraction

Total DNA was extracted from peripheral blood leucocytes using phenol-chloroform standard procedures [24].

2.2.2. Analysis of the GAA trinucleotide repeat expansion in the FXN gene

The expanded region in intron 1 of the *FXN* gene was amplified with classic and Long range PCR using GoTaq Long PCR Master Mix (M4021) (Promega). This last reaction was performed with 50 ng of genomic DNA and 4 ng/μL of each primer: 5′ATGGATTTCCTGGCAGGACGC-3′ for the forward primer and 5′GCATTGGGCG-ATCTTGGCTTAA-3′ for the reverse one. The conditions for the Long range PCR reaction were as follows: initial denaturation at 95 °C for 5 minutes, followed by

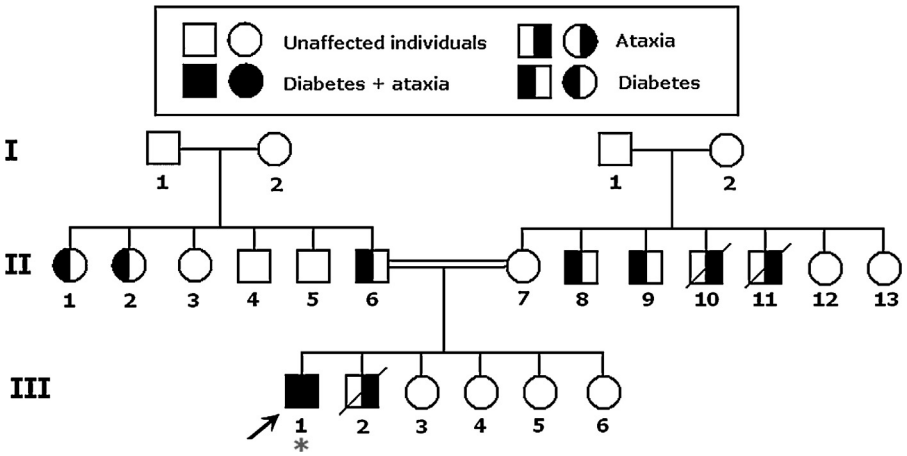

Fig. 1. Pedigree of the family of the Tunisian patient with Friedreich's ataxia. Asterisks indicate the individuals from whom DNA samples were obtained and tested. Generations are indicated on the left in Roman numerals and the numbers under the individuals represent identification numbers for each generation.