Mitochondrion 22 (2015) 17–22

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Mutation loads in different tissues from six pathogenic mtDNA point mutations

María M. O'Callaghan [a,c], Sonia Emperador [b,c], Mercè Pineda [a,c], Ester López-Gallardo [b,c], Raquel Montero [a,c], Delia Yubero [a], Cristina Jou [a], Cecilia Jimenez-Mallebrera [c,d], Andrés Nascimento [a,c], Isidre Ferrer [e], Angels García-Cazorla [a,c], Eduardo Ruiz-Pesini [b,c], Julio Montoya [b,c], Rafael Artuch *[a,c]

[a] Departamentos de Neurología, Bioquímica Clínica y de Patología, Hospital Sant Joan de Déu, Barcelona, Spain
[b] Departamento de Bioquímica, Biología Molecular y Celular, Universidad de Zaragoza, Instituto Aragonés de Ciencias de la Salud, Spain
[c] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Spain
[d] Unidad de Patologia Neuromuscular, Hospital Sant Joan de Deu, Barcelona, Spain
[e] Instituto de Neuropatología, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Centro de Investigación Biomédica de Enfermedades Neurodegenerativas (CIBERNED), Spain

ARTICLE INFO

Article history:
Received 7 November 2014
Received in revised form 30 January 2015
Accepted 3 March 2015
Available online 10 March 2015

Keywords:
Mitochondrial DNA point mutations
Mutation load
Urine
MELAS
Mitochondrial diseases

ABSTRACT

In this work, we studied the mtDNA mutations *m.3243A > G*, *m.3252A > G*, *m.15923A > G*, *m.13513G > A*, *m.8993T > G* and *m.9176T > C* in the blood, urine and buccal mucosa of a cohort of 27 subjects.
Urine cells had the highest mutation load for all of the mtDNA mutations studied. The mutation loads in the blood, urine and the buccal mucosa were significantly higher in the mitochondrial disorder group that manifested clinical signs than in the asymptomatic subjects. In conclusion, urine is a suitable biological sample for molecular diagnosis of mtDNA mutations and for the study of the attendant risk of recurrence in the offspring of asymptomatic mothers identified as non-carriers after mutation analysis in blood.

1. Introduction

Mitochondrial DNA (mtDNA) point mutations affect protein-coding or protein-synthesis machinery genes of the oxidative phosphorylation system (Scaglia and Wong, 2008; Zeviani and Di Donato, 2004; Zifa et al., 2007). These mutations cause a wide spectrum of clinical phenotypes with high transmission maternal inheritance risks (Monnot et al., 2011).

Pathogenic mtDNA point mutations usually co-exist with wild-type mtDNA in the same tissue, and the degree of heteroplasmy varies widely between individuals and also between tissues within the same subject (Chinnery et al., 1997). These facts seems to be the main factors responsible for the varied clinical expressions of mtDNA mutations, that include a diversity of clinical phenotypes involving different organs, severities and ages of the onset of disease (Chinnery et al., 1997; Ciafaloni et al., 1991; Holt et al., 1990; Macmillan et al., 1993; Matthews et al., 1994). Although muscle is the gold standard for the study of mtDNA mutation loads (muscle has the highest mutation load that remains invariable with time) ('t Hart et al., 1996), muscle biopsy is an invasive procedure that is difficult to perform routinely in clinical practice (Ma et al., 2009).

Previous reports have studied the *m.3243A > G* mutation loads in different accessible samples to identify a non-invasive and rapid diagnostic method that can identify patients with mitochondrial disorders (Chinnery et al., 1999; de Laat et al., 2012; Sue et al., 1998). These studies have shown that the *m.3243A > G* mutation load in urine is consistently higher than that in blood and the buccal mucosa (de Laat et al., 2012; McDonnell et al., 2004; Rahman et al., 2001; Shanske et al., 2004) and closely resembles the mutation load in muscle (de Laat et al., 2012; Frederiksen et al., 2006; McDonnell et al., 2004). The mutation load in urine decreases with age to a lesser extent than the mutation load in blood (Frederiksen et al., 2006; Rahman et al., 2001; 't Hart et al., 1996).

In this work, we present a family that harbored the *m.3243A > G* mutation, describe the clinical features of every member and examined the mutation loads in the blood, urine and buccal mucosa. Furthermore, we report the mutation loads of five different pathogenic mtDNA point mutations in the blood, urine and buccal mucosa in a series of cases and their relatives.

Abbreviations: DNA, mtDNA, mitochondrial; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MD, mitochondrial disease.

* Corresponding author at: Clinical Biochemistry Department, Passeig Sant Joan de Déu, 2, 08950 Esplugues, Barcelona, Spain. Tel.: +34932806169; fax: +34932803626.
E-mail address: rartuch@hsjdbcn.org (R. Artuch).

http://dx.doi.org/10.1016/j.mito.2015.03.001
1567-7249/

2. Material and methods

2.1. Patients

The index case for the *m.3243A > G* mutation was a woman (Fig. 1; case IV.6) who visited the Neurology Department of our Hospital. Due to her familial antecedents (one relative (Fig. 1; case III.1) was diagnosed with MELAS after necropsy), we studied her and 18 relatives including cases from previous generations. The other 6 index cases were Caucasian pediatric patients (2 boys and 4 girls) from 6 different families. We studied all of their mothers and, in 2 cases, also studied 2 siblings. The clinical and laboratory data are presented in Tables 1 and 2.

Regarding the clinical symptoms, all of the cases were scored and classified into 2 groups, asymptomatic patients ($n = 11$), and symptomatic patients ($n = 16$) (see Tables 1 and 2), based on the clinical signs and symptoms of the Morava's Mitochondrial Disease (MD) Criteria (Morava et al., 2006). The ages of all patients correspond to the times at which the biological samples were collected.

Considering all index cases and their relatives, we studied a total of 33 subjects including 8 males and 25 females whose ages ranged from 2 to 78 years (average 33.3 years; SD = 19.2). Biological samples (blood, urine and buccal mucosa) were available for 27 cases.

2.2. Samples

Blood samples were obtained by venous puncture, urine samples were obtained from spontaneous urination from the first morning void (minimum sample volume: 25 mL) and buccal mucosa cells were obtained by brushing the cheek in the oral cavity with a sterile stick brush. In 7 cases, muscle biopsies was performed and processed as previously reported (Montero et al., 2008).

2.3. Histopathological investigations

Open muscle biopsies of the deltoid were performed in cases I, III and IV, and the quadriceps muscle was biopsied in case V and her sister. The specimens were either frozen in isopentane or cooled in liquid nitrogen for histochemical analysis. The specimens from case III and the sister of case V were fixed in glutaraldehyde for electron microscopy. Serial frozen sections were stained with standard techniques for hematoxylin and eosin, modified Gomori trichrome, nicotinamide adenine dinucleotidtetrazolium reductase, succinate dehydrogenase and cytochrome c oxidase. A standard technique was applied for electron microscopy.

2.4. Laboratory studies

Genomic DNA was extracted from the different biological samples with standard procedures. Mitochondrial DNA was amplified by polymerase chain reaction using specific oligonucleotides primers that corresponded to each studied mutation. The percentages of mutations were analyzed by last-cycle radioactive polymerase chain reaction and restriction fragment length polymorphisms after cutting the amplified fragment with the specific restriction enzyme. The digested products were electrophoresed in agarose gels.

2.5. Statistical analyses

A Kolmogorov–Smirnov test was applied to study the data distribution. Because the data followed a Gaussian distribution, parametric Student's *T* tests for paired data analyses were used to compare the mutation loads in the blood, urine and buccal mucosa from the cases that harbored the different studied mutations. Pearson tests were applied to examine the correlation of the mutation loads in different tissues with the ages of the patients. Levene and Student's *T* tests were applied to compare the mutation loads in the blood, urine, buccal mucosa and saliva between the cases with symptomatic mitochondrial disorder and the cases with no symptoms or signs. The calculations were performed with the SPSS 20.0 program. Statistical significance was considered at $p < 0.05$.

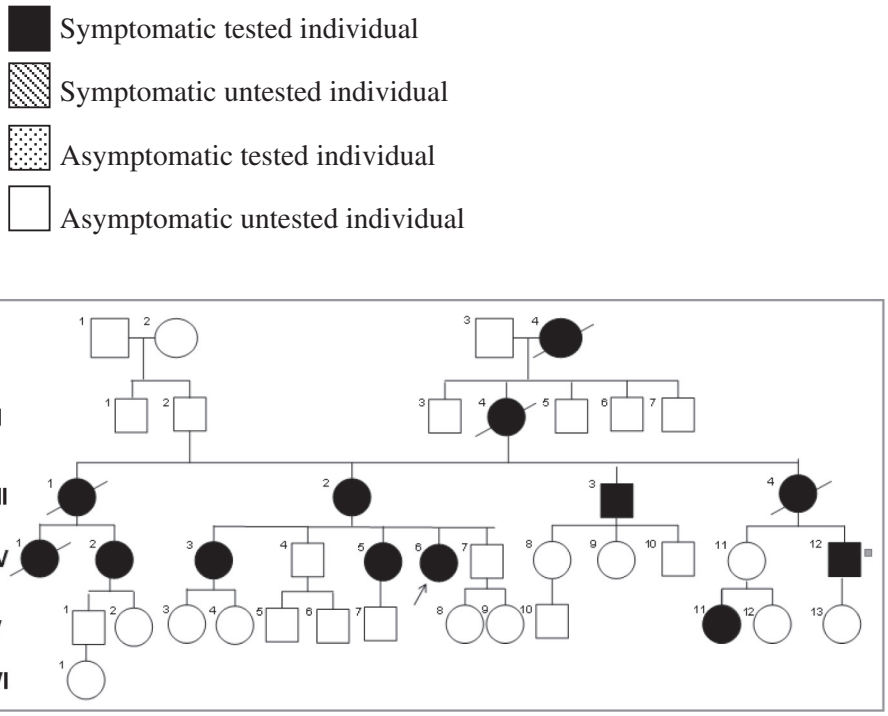

Fig. 1. Pedigree of *m.3243A > G* family. Symptomatic patients are in black. Arrow points index case.