Neurological Sciences (2019) 40:1705–1708
https://doi.org/10.1007/s10072-019-03864-w

BRIEF COMMUNICATION

Complex multisystem phenotype associated with the mitochondrial DNA m.5522G>A mutation

Claudia Nesti[1] · Anna Rubegni[1] · Deborah Tolomeo[2] · Jacopo Baldacci[1] · Denise Cassandrini[1] · Francesca D'Amore[1] · Filippo M. Santorelli[1]

Received: 17 December 2018 / Accepted: 21 March 2019 / Published online: 1 April 2019

Abstract

Mitochondrial tRNAs are responsible for more than half of pathogenic point mutations in the mitochondrial genome (mtDNA). Different mutations give rise to widely differing phenotypes, ranging from isolated organ-specific diseases to multisystem conditions. Herein, we report a 40-year-old woman presenting with a complex multisystem phenotype including sensorineural hearing loss, retinopathy, severe dilated cardiomyopathy, non-insulin dependent diabetes mellitus, and renal failure. Sequence analysis of mtDNA identified the m.5522G>A mutation in *MT-TW*, the gene encoding mitochondrial tRNA for tryptophan. The heteroplasmic variant, thus far described once, was almost exclusively confined to skeletal muscle tissue, as shown by massive parallel sequencing and corroborated by an ad hoc designed PCR-based strategy. This patient, presenting a severe, multisystem involvement apparently sparing the brain, contributes to the genetic heterogeneity of mitochondrial diseases caused by mutations in mitochondrial tRNAs.

Keywords mtDNA mutation · Heteroplasmy · $tRNA^{Trp}$ · Multisystem disorder · Massive parallel sequencing

Introduction

Even though mtDNA-encoded tRNA genes (mt-tRNAs) make up only around 5% of the total mitochondrial genome, 70–75% of all the gene mutations linked to mitochondrial pathologies are found in mt-tRNAs. This is explained by the essential role that mt-tRNAs play in mitochondrial protein synthesis and oxidative phosphorylation [1]. Mutations in mt-tRNAs are associated with extremely high clinical and allelic heterogeneity, and the spectrum of possible phenotypes is growing at an incredible rate. Any single report of an allegedly pathogenic new mutation needs to be followed by descriptions of further cases, eventually on a different genetic background, to allow more robust disease-associated correlations.

Herein, we present clinical and molecular findings in a woman who harbored a heteroplasmic mutation in the *MT-TW* gene. This is the second case reporting the m.5522G>A variant. Interestingly, in our patient, the gene mutation appeared to be almost exclusively confined to the skeletal muscle in spite of multisystem clinical involvement.

Materials and methods

All the procedures complied with the Helsinki Declaration of 1975. This study was approved by our institutional ethics committee.

Total DNA purification, genetic studies in tissues, procedures to perform muscle and skin punch biopsy, and spectrophotometric determination of the activities of respiratory chain (RC) enzyme complexes were performed with described methodologies [2] and with the patient's written informed consent. Whole mitochondrial genome sequencing was

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-019-03864-w) contains supplementary material, which is available to authorized users.

✉ Claudia Nesti
cla_nesti@yahoo.it

✉ Filippo M. Santorelli
filippo3364@gmail.com

[1] Molecular Medicine for Neurodegenerative and Neuromuscular Diseases Unit, IRCCS Stella Maris Foundation, via dei Giacinti 2, 56128 Pisa, Italy

[2] Department of Clinical and Experimental Medicine, University of Pisa, via dei Giacinti 2, 56128, Calambrone, Pisa, Italy

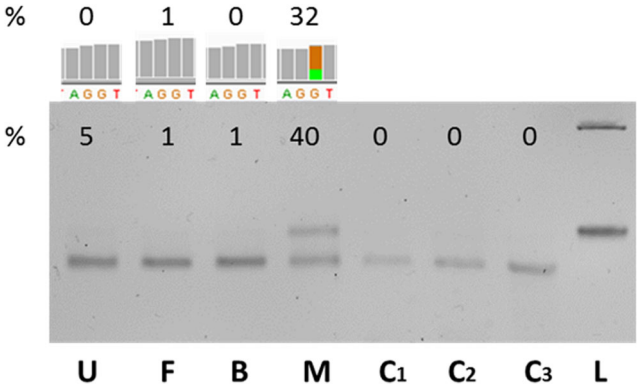

Fig. 1 Molecular analyses in a new patient harboring the m.5522G>A mtDNA mutation. Using a massive parallel sequencing approach and visualizing the coverage with IGV software (http://software.broadinstitute.org/software/igv/), we found 0–1% mutated mtDNA molecules in urine, fibroblasts, and blood from the proposita whereas the mutation load was above 30% in the skeletal muscle DNA (upper panel). The PCR-RFLP analysis (lower panel) showed that the proportion of mutant genomes varied greatly amongst different patient tissues. The endonuclease *Hph*I cleaves the wild type molecules in two fragments sized 80 and 16-base pairs (bp); the presence of the m.5522G>A removes a site of cleavage resulting in the uncut fragment of 96 bp. The heteroplasmic mutation results in fragments sized 96, 80, and 16 (not shown) bp. U, urine; F, fibroblasts; B, blood; M, skeletal muscle; C, control DNA; L, DNA molecular marker ladder

performed by massive parallel sequencing using NexteraXT technology (Illumina, San Diego, CA). Isolation of single muscle fibers was performed on serial 10-μm-thick transversal muscle sections stained for cytochrome *c* oxidase (COX) activity and prepared in two independent microdissection experiments using a laser capture microdissection (LCM) instrument (MMI Cell Cut, Nikon, Japan). An ad hoc designed PCR-RFLP strategy was used to quantify the abundance of the m.5522G>A in tissues and single muscle fibers (see legend to Fig. 1). Investigation of coding variants in a large set of nuclear DNA-encoded mitochondrial genes was performed in blood DNA from the proposita with targeted massive gene sequencing as we described elsewhere [3, 4].

Results

Case description

This patient is a 40-year-old woman, born to healthy non-consanguineous parents, presenting with a complex multisystem disorder. Her family history was remarkable for a younger brother who had died at the age of 2 months of an unspecified heart malformation. Early development, neonatal, and school-age periods were unremarkable. Since adolescence, the patient reported sensorineural hearing loss and retinopathy. She subsequently developed non-insulin dependent diabetes mellitus, hypothyroidism, and ovarian failure. At age 33, she presented heart failure and an echocardiogram disclosed severe dilated cardiomyopathy. At age 39, a defibrillator device was implanted. Moreover, the patient presented episodes of easy fatigue. Neurological examinations and neuropsychological evaluations were within normal limits, however. A routine blood test showed signs of renal and liver failure while serum creatine kinase levels were within normal limits. At age 43, an abdominal ultrasound exam showed findings suggestive of cirrhotic liver. Metabolic assessments revealed repeatedly high serum alanine levels (on average 2× normal values). Brain MRI was not performed because of the defibrillator. An EMG study did not reveal findings suggestive of a myopathy. Despite the progressive multisystem worsening the neuromuscular assessment remained stable over 4-years follow-up.

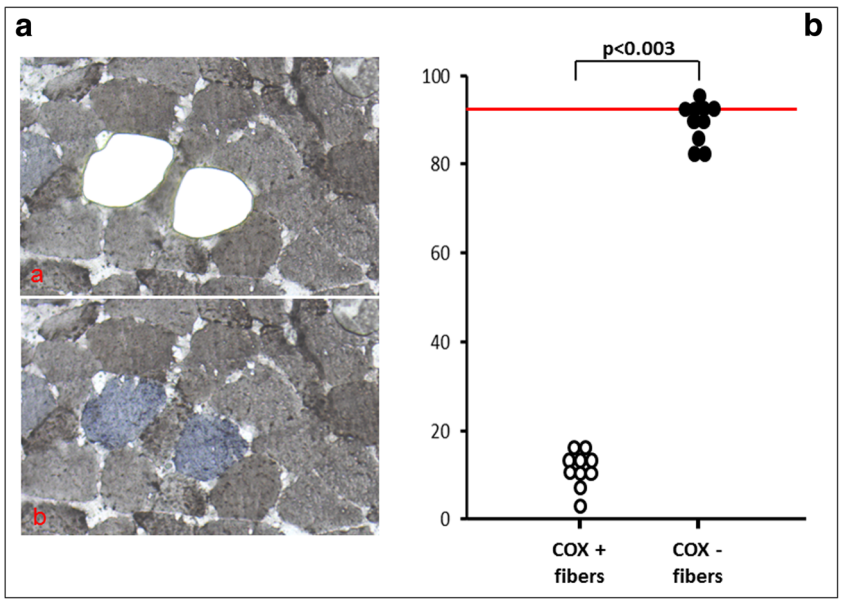

Fig. 2 Single-fiber PCR analysis for m.5522G>A mutation. **a** Micro-dissected muscle tissue sections. COX− fibers selected for studies are shown before (b) and after (a) laser capture microdissection (LCM). The same procedure was performed for COX+ fibers. **b** Graphical representation of the levels of m.5522G>A mutation in COX+ ($n = 10$) and COX − fibers ($n = 10$), the latter showing a significantly higher rate of mutant load. Student's *t* test, $p < 0.003$

Laboratory investigations

The muscle histochemistry detected about 10% of muscle fibers to be COX negative (COX−), succinate dehydrogenase positive (SDH+). RC enzyme analyses showed that the activity of complex I was moderately reduced with values being at the lower normal limit (0.07 mmol/min/g tissue; normal range 0.07–0.18). While a preliminary Sanger sequencing test in blood DNA was normal, we later used massive mtDNA sequencing in muscle DNA and identified the heteroplasmic m.5522G>A mutation in *MT-TW*, the gene encoding mt-tRNA for tryptophan (Fig. 1). An ad hoc designed PCR-based strategy showed the mutation to be present in 40% of mitochondrial genomes in muscle and 5% in urinary sediments, whereas it was barely detectable in the blood, skin fibroblasts, and buccal swab from the patient, or in blood and urine from her healthy mother. No other maternal relatives were investigated. Single muscle fibers showed the highest levels of mutant genomes (98%) in COX−, whereas COX+ fibers harbored an average of 15% mutant mtDNAs (Fig. 2). Targeted gene panel investigating the coding exons of over 200 mitochondrial nDNA-encoded genes failed to detect rare pathogenic variants (MAF < 0.01) of predictable clinical significance (Supplementary Table lists variants of unknown significance).

Discussion

Most mt-tRNA mutations have only been described in single kindred and appear to be private raising doubts on their real disease-related association. The m.5522G>A mutation in *MT-TW* was found once in a patient with mild muscle symptoms, high creatine kinase levels, and histopathological findings of mitochondrial myopathy. In that case, other tissues, including blood, harbored few mutant mtDNAs [5]. In our proband, presenting a severe multisystem involvement apparently sparing the brain, the association of cardiac, renal, and endocrinological features further expands the clinical presentation of the m.5522G>A mutation. This variant satisfies commonly accepted criteria for pathogenic mtDNA variants, and it is scored as "definitely pathogenic" by applying MitoTIP [6] and more standard tools to assess pathogenicity of mt-tRNA mutations [7].

Eighteen mutations have been thus far described in *MT-TW*, all presenting a wide array of clinical phenotypes characterized by predominant neurological manifestations and ranging from Leigh-like features to isolated myopathy [8]. This further supports the suggestion of the mt-tRNA gene for tryptophan as an additional hot spot for mutations. As recently reported in our revision of the pertinent literature [8], differences in clinical presentation between patients appear unrelated to mutant mtDNA load or location in the cloverleaf structure of the tRNA; therefore, other genetic or epigenetic factors should be invoked.

Two additional remarks are worth mentioning in our case. First, our report highlights the importance of performing whole mitochondrial genome analysis on DNA purified from skeletal muscle, even when muscle symptoms appear overshadowed by a multisystem presentation. This might also be important when blood mtDNA tests remain mute in spite of a highly suggestive phenotype. Second, and more important, massive parallel sequencing techniques should be preferred for testing mtDNAs, as they make it possible to detect even tiny amounts of mutant molecules and appraise heteroplasmic variants more accurately. This is important to avoid the false impression of negative results as our early blood test in the proband.

Acknowledgments We are indebted to Dr. Catherine J. Wrenn for expert editing of the manuscript and critical advice.

Funding information This work was partially supported by grant MITONEXT (to FMS) and Ricerca Corrente 5x1000 2018 from the Italian Ministry of Health.

Compliance with ethical standards All the procedures complied with the Helsinki Declaration of 1975. This study was approved by our institutional ethics committee.

References

1. Tang S, Wang J, Zhang VW, Li FY, Landsverk M, Cui H, Truong CK, Wang G, Chen LC, Graham B, Scaglia F, Schmitt ES, Craigen WJ, Wong LJC (2013) Transition to next generation analysis of the whole mitochondrial genome: a summary of molecular defects. Hum Mutat 34:882–893
2. Cassandrini D, Savasta S, Bozzola M, Tessa A, Pedemonte M, Assereto S et al (2006) Mitochondrial DNA deletion in a child with mitochondrial encephalomyopathy, growth hormone deficiency, and hypoparathyroidism. J Child Neurol 21:983–985
3. Rubegni A, Ferrari AR, Pasquariello R, Canapicchi R, Santorelli FM, Nesti C (2018) Relapsing-remitting course of cystic leukoencephalopathy. Pediatr Neurol 89:63–65
4. De Michele G, Sorrentino P, Nesti C, Rubegni A, Ruggiero L, Peluso S et al (2018) Reversible valproate-induced subacute encephalopathy associated with a MT-ATP8 variant in the mitochondrial genome. Front Neurol 9:728
5. Barić I, Fumić K, Petković Ramadža D, Sperl W, Zimmermann FA, Muačević-Katanec D, Mitrović Z, Pažanin L, Cvitanović Šojat L, Kekez T, Reiner Ž, Mayr JA (2013) Mitochondrial myopathy associated with a novel 5522G>A mutation in the mitochondrial tRNA(Trp) gene. Eur J Hum Genet 21:871–875

6. Sonney S, Leipzig J, Lott MT, Zhang S, Procaccio V, Wallace DC, Sondheimer N (2017) Predicting the pathogenicity of novel variants in mitochondrial tRNA with MitoTIP. PLoS Comput Biol 13: e1005867. https://doi.org/10.1371/journal.pcbi.1005867
7. Yarham JW, Al-Dosary M, Blakely EL, Alston CL, Taylor RW, Elson JL et al (2011) A comparative analysis approach to determining the pathogenicity of mitochondrial tRNA mutations. Hum Mutat 32:1319–1325
8. Cardaioli E, Mignarri A, Cantisani TA, Malandrini A, Nesti C, Rubegni A, Funel N, Federico A, Santorelli FM, Dotti MT (2018) Myoclonus epilepsy, retinitis pigmentosa, leukoencephalopathy and cerebral calcifications associated with a novel m.5513G>A mutation in the MT-TW gene. Biochem Biophys Res Commun 500:158–162