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Early onset cardiomyopathy associated with the mitochondrial tRNALeu(UUR) 3271T>C MELAS mutation

Giacomo Brisca [a], Chiara Fiorillo [b], Claudia Nesti [b], Federica Trucco [c], Maria Derchi [d], Antonio Andaloro [e], Stefania Assereto [c], Guido Morcaldi [a], Marina Pedemonte [c], Carlo Minetti [c], Filippo M. Santorelli [b, **], Claudio Bruno [a, *]

[a] Center of Myology and Neurodegenerative Disorders, Istituto Giannina Gaslini, Genova, Italy
[b] Neuromuscular and Molecular Medicine Unit, IRCCS Stella Maris Foundation, Pisa, Italy
[c] Pediatric Neurology Unit, Genova, Italy
[d] Pediatric Cardiology Unit, Genova, Italy
[e] Orthopaedics and Traumatology Unit, Istituto Giannina Gaslini, Genova, Italy

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ABSTRACT

Mitochondrial disorders are a heterogeneous group of diseases sharing a defect of the oxidative phosphorylation system.

Point mutations in the mitochondrial DNA are a common cause of mitochondrial disorders and frequently affect the sequences encoding mitochondrial transfer RNAs.

The m.3271T>C mutation in the mitochondrial $tRNA^{Leu(UUR)}$ is traditionally reported in patients with clinical features of the mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome and in mitochondrial diabetes.

Here we describe the clinical, pathological, and molecular features of an Italian child and his asymptomatic mother, carrying the m.3271T>C mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene, in association with an unusual clinical phenotype dominated by hypertrophic cardiomyopathy and provide review literature of cases with this mutation.

To the best of our knowledge, there are no reports describing the association of this mutation with cardiomyopathy, and our cases suggest that the m.3271T>C mutation has to be taken into account in the diagnostic approach of maternally inherited cardiomyopathies.

1. Introduction

Mitochondrial respiratory chain disorders are a heterogeneous group of diseases that share the involvement of the cellular bioenergetic machinery affecting the mitochondrial oxidative phosphorylation system (OxPhos). They usually present as multisystem disorders but the nervous system and skeletal muscles are mainly involved [1].

Point mutations in the mitochondrial DNA (mtDNA), the most common genetic cause of mitochondrial respiratory chain disorders, are associated with a wide spectrum of pathological phenotypes; most of them affect mitochondrial transfer RNA (mt-tRNA) genes especially $tRNA^{Leu(UUR)}$, $tRNA^{Ile}$, and $tRNA^{Ser(UCN)}$. Pathogenic tRNA mutations typically impair mtDNA translation, resulting in the disruption of protein synthesis and causing a generalized reduction of the activities of the respiratory chain complexes containing mtDNA-encoded polypeptides. Currently, more than 270 pathogenic point mutations in mtDNA are listed in the MITOMAP database (www.mitomap.org), and approximately half of which map to tRNA genes [2]. However, no strict correlations between mutation and clinical phenotype have been shown.

Point mutations in the mt-$tRNA^{Leu(UUR)}$ gene are traditionally reported in patients with the mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome

* Corresponding author. Center of Myology and Neurodegenerative Disorders, Istituto Giannina Gaslini, Largo G. Gaslini, 5 – 16147 Genova, Italy. Fax: +39 010 8612070.
** Corresponding author.
E-mail addresses: filippo3364@gmail.com (F.M. Santorelli), claudiobruno@ospedale-gaslini.ge.it (C. Bruno).

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phenotype, the most common mtDNA-related disorder, with the vast majority of patients harbouring the A-to-G transition at nucleotide 3243 (m.3243A>G), whereas approximately 10% of patients carry m.3271T>C [3].

Here, we describe the clinical, pathological and molecular features of an Italian boy and his mother in which the m.3271T>C results in an atypical clinical phenotype.

To our knowledge this is the first report that detects the association between this “classical” MELAS mutation and hypertrophic cardiomyopathy.

2. Material and methods

2.1. Patient 1

BM is a 17-year-old boy, the only child of non consanguineous parents. He was born from an uneventful full-term pregnancy. His psychomotor developmental milestones were within normal range.

Since age 3 years he started complaining easy fatigability and myalgia and showed a severe failure to thrive. Otherwise clinical history was negative; in particular he did not complain of headache nor seizures.

At clinical examination at age 13, when the boy was first referred to our hospital, the patient was extremely small for his age with height and weight below the third centile. Blood pressure was normal. Endocrinological evaluation detected a complete growth hormone deficiency and replacement treatment was started.

Neurological assessment documented generalized hypotonia, wasting and weakness with positive Gowers sign. His tendon reflexes were ubiquitously reduced.

Extensive laboratory blood testing was performed: CK was mildly increased (637 U/l with normal values < 150). Serum lactate was elevated (65.1 mg/dl, n.v. < 20). Serum glycemia was normal. No additional abnormalities were detected.

Electromyography showed a myopathic pattern. Nerve conduction studies were in the normal range. X-ray detected a generalized osteoporosis with epiphyseal abnormalities of the knees In addition bone age resulted severely delayed. Audiometric examinations demonstrated a mild sensory-neural hearing-loss.

A complete cardiac examination was also performed: electrocardiography (EKG) showed sinus rhythm, normal heart rate, and signs of left ventricular hypertrophy. Accordingly, echocardiography documented mild concentric hypertrophic cardiomyopathy showing increased left ventricular mass index with increased size of ventricular septum and posterolateral wall of left ventricle (IVSd 14 mm, Z-score 7.11; LVPWd 15 mm, Z-score 6.23). Cardiac chambers had normal dimensions and sisto-diastolic function was normal (shortening fraction 34%; ejection fraction 60%; LVEDD 49 mm, Z-score 2.00). EKG Holter monitoring did not show arrhythmia.

Electroencephalography (EEG), brain MRI and MR-spectroscopy were unremarkable.

Muscle MRI with axial T1-weighted sequences showed a generalized hypotrophy without fatty infiltration in the thighs.

The patient underwent a muscle biopsy from vastus lateralis. Histochemically, we observed increased size variability with presence of hypotrophic muscle fibers, several ragged red fibers (RRF) which resulted to be negative with the cytochrome *c* oxidase (COX) staining, whereas the same fibers appeared strongly stained using the succinate-dehydrogenase (SDH) technique (Fig. 1). Furthermore, several muscle fibers displayed increased lipid droplets at the Oil red O (ORO) staining.

Spectrophotometric analyses of the respiratory chain enzyme complexes showed a generalized reduction of all complexes activity.

Molecular analyses of mtDNA ruled out the presence of large-scale deletions and depletion whereas it identified the m. 3271T>C mutation in mt-tRNA$^{Leu(UUR)}$.

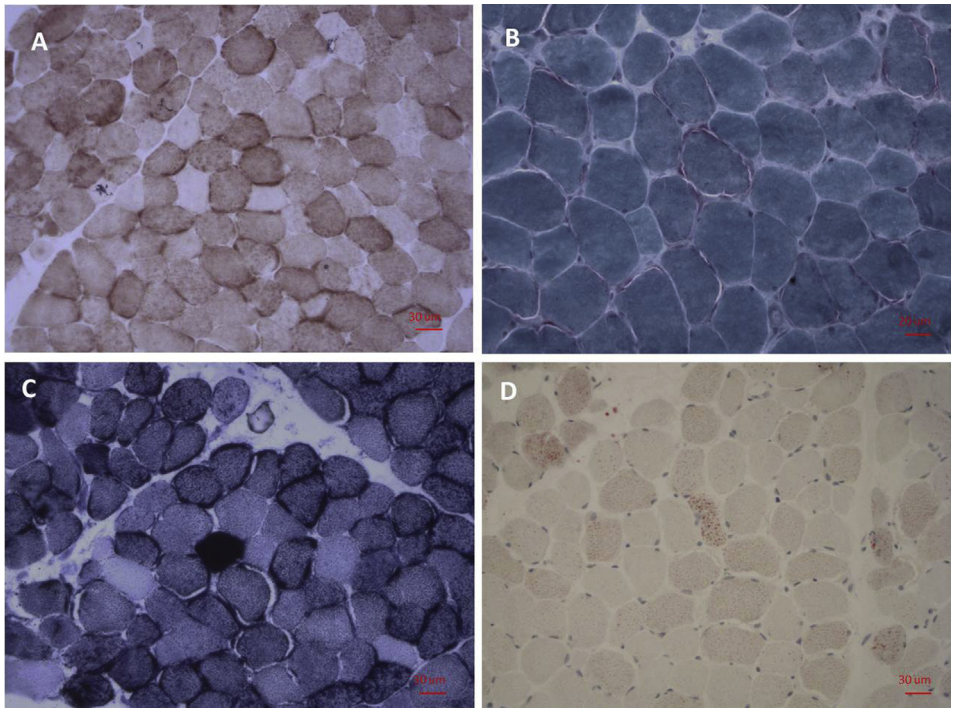

Fig. 1. Muscle biopsy of patient 1. A. With COX staining several COX negative fibers are evident. B. Ragged red fibers are shown with Gomori Trichrome. C. Increased SDH staining is present in COX negative fibers. D. Oil Red O staining displays several fibers with increased lipid droplets. A, C, D, 10X magnification and B, 20X magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

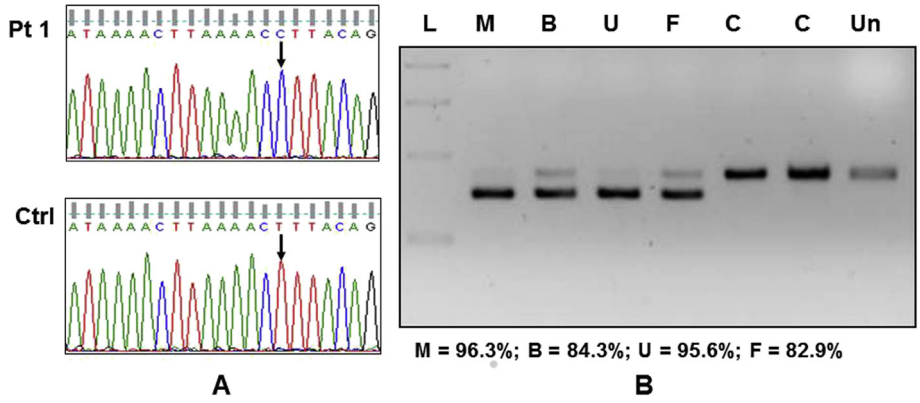

Fig. 2. Molecular studies. A. Sequence chromatograph of the tRNA$^{Leu(UUR)}$ region flanking the m.3271T>C mutation (arrow) in skeletal muscle from our patient as compared with a wild type control (Ctrl). B. PCR-Restriction Fragment Length Polymorphism analysis showing the presence of the m.3271T>C mutation. The restriction endonuclease *Afl*II cleaves mutated sequences in two fragments (sized 142- and 30-bp, the latter is not visible in the figure) whereas wild-type sequences remain uncut (172bp). L: 100-bp DNA ladder; M: muscle; B: blood; U: urine; F: fibroblasts; C: control; Un: uncut.

The mutation load was 84.3%, 96.3%, 95.6% and 82.9% in blood, muscle, urine and fibroblasts, respectively (Fig. 2).

2.2. Patient 2

RI is the 37-year-old asymptomatic mother of patient 1. She was full term born with a normal psychomotor development. She had never complained of myalgia nor exercise intolerance and had had not history of stroke episodes, migraine or seizures.

On clinical examination at age 37, her stature and weight were in the normal range; she showed normal muscular tone, bulk and strength. Tendon reflexes were normal. She had no visual nor hearing disturbances.

Blood tests showed increased levels of lactate (47.2 mg/dl, with normal values 8–22) with a very mild metabolic acidosis. CK and glycaemia were in the normal range.

Echocardiography showed a mild hypertrophic cardiomyopathy involving only the left ventricle, with the interventricular septum thickness of 12 mm (normal value $\leq$12 mm). The m.3271T>C was 21.6%, 51% and 71.6% in blood, urine and fibroblasts, respectively.

3. Discussion

Although over 270 pathogenic mtDNA mutations have been reported to date, the genetic etiology of a mitochondrial disease is not straightforward. MtDNA mutations are located across the 37 genes of the mitochondrial genome but the vast majority resides in just 5–10% of the mtDNA, such as in the 22 mt-tRNAs. In particular, the mt-tRNA$^{Leu(UUR)}$ gene is a recognized mutations hot spot and currently more than 20 pathogenic point mutations have been reported in this gene. Associated clinical phenotypes are heterogeneous and include MELAS, myoclonic epilepsy and ragged red fibers (MERRF), chronic progressive external ophtalmoplegia (CPEO),

Table 1
Characteristics of patients with tRNA$^{Leu(UUR)}$ m.3271T>C mutation.

Reference	Epilepsy	Migraine	Stroke	Cardio myopathy	Growth failure	Myopathy	Lactic acidosis	Deafness	Diabetes	Other	Brain MRI	Muscle biopsy	Mutation load (%) M/B/F/U
Marie et al., 1994 [6]	1/1	0/1	1/1	0/1	0/1	1/1	1/1	NA	0/1	–	NA	RRF	NA
Goto et al., 1995 [10]	NA	NA	3/3	0/3	3/3	3/3	3/3	NA	NA	–	NA	NA	NA
Suzuki et al., 1996 [7]	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	Neuropathy	NA	NA	NA
Tarnopolski et al., 1998 [4]	1/6	3/6	1/6	0/6	0/6	5/6	6/6	6/6	0/6	Neuropathy	NA	RRF	95/11-51/ NA/NA
Shinde et al., 2000 [11]	1/1	NA	1/1	0/1	0/1	1/1	1/1	0/1	0/1	Rhabdomyolisis	Ischemic lesions	RRF	NA
Nagashima et al., 2001 [8]	3/3	0/3	0/3	0/3	0/3	3/3	3/3	2/3	0/3	MERRF-like, polyneuropathy	NA	RRF	NA
Barisic et al., 2002 [12]	1/1	NA	1/1	0/1	0/1	1/1	1/1	0/1	0/1	Spinal dysraphism	Hemorragic lesion	Normal	NA
McPherson et al., 2005 [9]	0/1	1/1	1/1	0/1	1/1	0/1	1/1	NA	0/1	Artrogryposis	Posterior infarct	NA	NA
Stenqvist et al., 2005 [13]	0/1	0/1	1/1	0/1	1/1	1/1	1/1	0/1	0/1	WPW disease	Cerebral atrophy	RRF	94/NA/94/NA
Tay et al., 2005 [3]	2/17	11/17	0/17	0/17	4/17	5/17	NA	1/17	1/17	–	WM lesions (1/17)	No RRF	NA/<5-31/ NA/<5-98
Chou et al., 2008 [14]	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	0/1	–	Lacunar infarction	RRF	100/77/NA/87
This report	0/2	0/2	0/2	2/2	1/2	1/2	2/2	1/2	0/2	Skeletal dysplasia	Normal (1/2)	RRF	96/21-84/ 71-82/51-95

References are indicated. NA: not available. RRF: ragged red fibers. M/B/F/U: muscle, blood, fibroblasts, urine. WM: white matter. WPW: Wolf-Parkinson-White.

diabetes mellitus, cardiomyopathy, deafness, and Leber hereditary optic neuropathy (LHON).

The most common mutations are the m.3243A>G and m.3271T>C. The latter affects the anticodon stem region of tRNA, which is not as highly conserved in evolution and does not interfere with transcription termination. However, cybrids obtained from HeLa cells and fibroblasts from a patient carrying the m.3271T>C showed impaired protein synthesis and decrease activity of the respiratory chain complex I [4].

Since the first description of m.3271T>C mutation in three patients affected by MELAS [5], less than 40 cases have been reported and most of them manifest the features associated with a MELAS phenotype (Table 1). Nevertheless, it has been suggested that patients could exhibit a milder and more heterogeneous phenotype than those harbouring the m.3243A>G mutation [6]. To date, however, there is a limited number of reports on atypical phenotypes associated with the m. 3271T>C (Table 1). In particular Suzuki et al., in 1996 described a patient carrying m.3271T>C mutation who exhibited only diabetes, without clinical evidence of muscle, heart or brain involvement [7].

Nagashima and colleagues in 2001 described three patients who presented a MERRF phenotype associated with a distinctive peripheral neuropathy [8]. Later on, Mc Pherson et al., in 2005 reported a girl affected by distal arthrogryposis at birth who subsequently developed a MELAS phenotype; the relationship between the mutation and distal arthrogryposis however remained uncertain [9].

In this work, we presented a child carrying the m.3271T>C mutation in association with a complex clinical picture, dominated by hypertrophic cardiomyopathy and marked lactic acidosis.

Lactic acidosis represents a constant feature in most of patients harbouring the mutation: Tarnopolski et al., in 1997, found an elevated resting plasma lactate concentration in 83% of patients, suggesting that the elevated resting lactate concentration was likely to be due to the metabolic block in several OxPhos complexes (I and IV primarily), present in 67% of patients [4]. Indeed in our patient we could demonstrate a multiple defect of respiratory chain complex activities.

Similarly, heart involvement is a typical finding in mitochondrial diseases and it has been associated with an ample array of different mutations in mitochondrial tRNAs including a handful in the $tRNA^{Leu(UUR)}$ gene [1]. Conversely there are only anecdotal reports of patients with m.3271T>C mutation and cardiomyopathy findings. Tarnopolski and collaborators in 1997 reported a kindred including two patients with suspected hypertrophic cardiomyopathy at autopsy, but more specific clinical and genetic data were not available [4]. The patient described in the present work presented overt signs of hypertrophic cardiomyopathy by age 13. In addition our patient presented a peculiar knees X-ray alterations resembling epiphyseal skeletal dysplasia. To our knowledge there are not clear association between skeletal dysplasia and mtDNA mutations and we are unable to link unquestionably bone alterations with deficient OxPhos function.

Finally, we confirmed in our mother and son family that there is no clear correlation between mutation load and clinical severity as suggested in Tay et al. [3]. Authors investigated the mutation load in different tissues from a large series of patients and detected that several asymptomatic patients carried mutant genomes, whereas no mutant genomes were depicted in selected clinically symptomatic patients [3]. We detected in patient 1 high level of mutant genomes in all investigated tissues (muscle, urine, blood and skin) and we also found a variable mutation load in the mother's fibroblast, urine and peripheral blood DNA although she is clinically asymptomatic.

In conclusion our cases add new data to the clinical spectrum of the m.3271T>C in $tRNA^{Leu(UUR)}$, suggesting that this mutation has to be taken into account in the diagnostic approach of maternally inherited cardiomyopathy.

Conflict of interest

None.

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Transparency document

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