A novel mtDNA point mutation in tRNA^{Val} is associated with hypertrophic cardiomyopathy and MELAS

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Hypertrophic cardiomyopathy; MELAS; mtDNA.

Background. **Pathological mutations of mitochondrial (mt) DNA may cause specific diseases such as cardiomyopathies or hearing loss, or syndromes such as mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode (MELAS) syndrome. We describe a novel mtDNA mutation in a patient with severe hypertrophic cardiomyopathy associated with MELAS. The familial phenotype included 1) hypertrophic cardiomyopathy and MELAS, 2) clinically mild cardiac hypertrophy, and 3) deafness.**

Methods. **The proband and her first degree relatives underwent echo and electrocardiograms, and biochemical tests. Magnetic resonance imaging of the brain was performed in the proband. mtDNA was fully analyzed by sequencing. DNA purification, polymerase chain reaction and direct automated sequencing were performed following standard procedures. Heteroplasmy of the novel mutation was quantified by densitometric analysis.**

Results. **A novel G1644A transition affecting the tRNA^{Val} was identified in the proband and maternal relatives. The mutation has been interpreted as pathological because the G at the 1644 position is a highly conserved base, is heteroplasmic with higher levels of mutant DNA in the proband than in the relatives, is located in the unique tRNA^{Val}, is very close to a mutation described as causative of MELAS, and finally has not been found in 100 healthy controls.**

Conclusions. **Although it is rare for patients with MELAS to be referred to cardiological evaluation because of coexisting cardiomyopathy, cardiologists should be aware of this association as well as of the non cardiac signs that may address the diagnosis to mtDNA defect-related disease in families with a variable phenotype.**

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Introduction

Hypertrophic cardiomyopathy (HCM) is causally linked to mutations in nuclear genes encoding structural or regulatory proteins of the sarcomere[1] and, less frequently, to mutations in mitochondrial (mt) DNA genes[2]. To date, many mtDNA mutations associated with the mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode (MELAS) syndrome have been identified. They include:

- ribosomal RNA/transfer (t) RNA transitions at 583[3], 1642[4], 3093[5], 3243[6-10], 3252[11], 3256[12,13], 3271[13], 3291[14] and 4317[15] bp;
- coding region transitions at 3308[16-18], 9957[19], 11084[20,21], 12147[22], 13513[23,24], 13514[25] and 14453[26] bp;
- a 4 bp deletion extending from the 14787 to 14790 nucleotide positions[27].

Two of the former tRNA defects, the G3243A[6-10] in the tRNA$^{LEU\ (UUR)}$ and the A4317G[15] in the tRNA^{Ile}, have been causally linked to MELAS with HCM.

Due to the peculiar features of mtDNA, a single point mutation in a mt-tRNA may cause severe organ/tissue disease. In fact, the protein synthesis apparatus of mitochondria works under extremely economic conditions since mtDNA codes for both rRNA subunits and for 22 tRNAs. This implies that even a single point mutation in a key position of a mtDNA-coded RNA may severely impair all the mitochondrial protein synthetic systems reflecting its negative effect on 4 out of 5 mitochondrial complexes (complexes I, III, IV and V) that are partly made up of polypeptides synthesized in the mitochondria. Furthermore, mtDNA-related diseases are complicated by the maternal inheritance of mtDNA genes and

their pleiotropic effect, incomplete penetrance, variable expression and heteroplasmy, i.e. the coexistence in the same tissue/cells of both mutated and wild type mtDNA at variable percentages. Thus, an identical mutation may cause different pathological phenotypes in different subjects within the same family, ranging from very mild clinical phenotypes to fully expressed diseases. Stochastic distribution to daughter cells of mitochondria containing normal and/or mutated mtDNA molecules plays a fundamental role in at least some unique pathological mtDNA features making it difficult: i) to foresee the percentage of affected molecules transmitted from cell to cell in a given tissue, ii) to infer, on the basis of the quantity found in other tissues, the amount of mutated mtDNA molecules of a tissue, and finally, iii) to establish reliable rules for genetic counseling.

In the present study, we report a novel pathological mtDNA mutation in the $tRNA^{Val}$ in a family in which the proband was diagnosed with HCM and MELAS, her mother with mild hypertrophy and both mother and grandmother with sensorineural deafness.

Methods

Family members and controls. The proband and her relatives were clinically investigated at the Cardiology Division of the University of Modena (Italy). They underwent interview, physical evaluation, electrocardiography, echocardiography and biochemical tests including serum creatine phosphokinase. Magnetic resonance imaging of the brain was performed on the proband. Controls for mtDNA study consisted of 100 healthy Italian Caucasoid subjects.

Amplification and nucleotide sequence determination. DNA was purified by a standard phenol-chloroform method. Polymerase chain reaction amplification was performed by using 200 ng of total DNA in accordance with standard protocols. The entire mtDNA molecule was amplified by polymerase chain reaction and sequenced as previously described[28]. For the region including the $tRNA^{Val}$ gene, forward 5'-TTAACTAAAACCCCTACG-CA-3' and reverse 5'-CGCCTATACTTTATTTGGGT-3' primers were used. Direct automated sequencing was carried out on amplified fragments in a 3100 ABI PRISM genetic analyzer (Applied Biosystem, Foster City, CA, USA). The mutated amplicons, obtained with the mismatch primer 5'-GCGGTCAAGTTAAGTTGAGAT-3' (nucleotide position 1647 underlined), were digested with the *Mbo*I restriction enzyme.

Analysis of the nuclear genes causally linked to HCM was not performed on the basis of clinical indications of a mtDNA-related disease in the proband.

Heteroplasmy quantification. The heteroplasmy of the G1644A mutation was quantified by densitometric analysis with a Gel Doc 2000 BIO-RAD instrument (Hercules, CA, USA).

Results

A novel mtDNA mutation was identified in a family (Fig. 1A) in which the phenotype ranged from mild left ventricular hypertrophy (II-2), cystic kidney disease (II-3, II-4, II-5), deafness (I-2, II-2), the absence of any clinical phenotype at the time of clinical evaluation (III-1) and severe HCM with MELAS (III-2).

Clinical data. A 33-year-old female (Fig. 1A, III-2) was referred for cardiological evaluation because of recent-onset dyspnea, peripheral edema and weight loss. At entry, she was diagnosed with HCM and pericardial effusion. Electrocardiography showed left cardiac hypertrophy and ventricular pre-excitation with a Kent branch. Echocardiography showed a left atrium at the upper limits of normal and left ventricular hypertrophy with a mean diastolic thickness of 3.6 cm and without a significant left ventricular outflow tract obstruction neither in basal conditions nor after provocative maneuvers (Valsalva and sublingual nitrate). No systolic anterior movement of the mitral valve apparatus was therefore detectable. The right ventricular wall was also hypertrophic. The left ventricular ejection fraction was decreased (35%), and there was a diastolic pattern of abnormal relaxation. All the hypertrophied myocardium showed a typical sparkling echo-texture which is a well-known ultrasonic feature of myocardial fiber disarray. Biochemical analysis documented increased levels of serum creatinine (3.6 mg/dl), glucose (146 mg/dl) and serum creatine phosphokinase (380 U/l). Other clinical features included mental retardation, muscular ataxia, sensorineural hearing loss and cystic renal disease. Brain magnetic resonance imaging showed widespread cerebral atrophy with secondary enlargement of the subarachnoid cistern. The patient was diagnosed with HCM associated with MELAS syndrome.

Family members (Fig. 1A): the mother (II-2), father (II-1) and brother (III-1), underwent clinical evaluation and echocardiographic screening. The mother was diagnosed with sensorineural deafness and mild cardiac hypertrophy (left ventricular thickness 1.2 cm). The brother showed normal echocardiographic data and no signs of hearing loss. Both mother and maternal grandmother were affected by sensorineural hearing loss and two maternal uncles and one aunt (not available for evaluation) were described as being affected by cystic renal disease. The father had normal echocardiographic findings. The family environment appeared to be quite critical, with conflicts among members that prevented the extension of the clinical and molecular evaluation to all living relatives, in particular to the maternal uncles.

At 12 and 18 months of follow-up the patient was clinically stable with a creatinine value of about 2.8 mg/dl and no dyspnea. The possibility of a double transplantation of the kidney and heart was excluded

because of the very high risk associated with such surgery and in view of the patient's clinical conditions, mental disorder and the critical family environment.

Molecular data. mtDNA of the proband was fully sequenced and showed a novel G1644A transition affecting the $tRNA^{Val}$ (Fig. 1 B, C and D), besides A2706G, T14766C, T16086C non-pathological mutations. The G1644A transition was present in the DNA of the proband's mother and brother. The mutant mtDNA was quantified as 85.4, 76 and 74.1% in the total DNA from the peripheral blood DNA of the proband, mother and unaffected brother, respectively. Heteroplasmy, evaluated using densitometric analysis, is shown for subjects II-2, III-1 and III-2 in figure 1C (lanes 2, 3 and 4): two bands of 148 and 124 bp are shown indicating the presence of mutated and normal mtDNA molecules.

Discussion

The present family highlights the complex clinical problems that may be associated with mtDNA defects: 1) the phenotype heterogeneity: different organs and tissues may be affected in the same patients or members of the same family. Cardiomyopathy may occur in patients with MELAS[6-10,15], but it may also represent the only clinically relevant phenotype in patients with

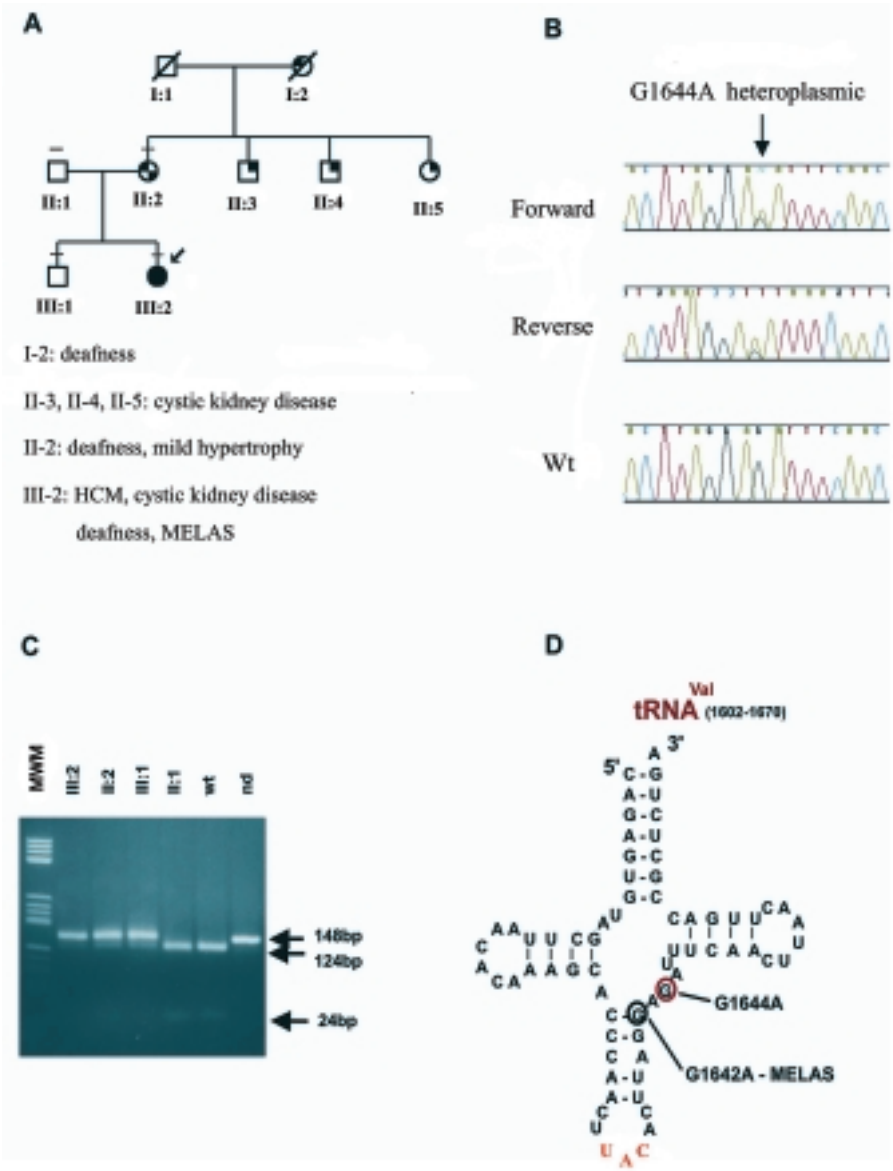

Figure 1. A: *family tree. Full black = affected proband; upper left black quarter = deafness; upper right black quarter = cystic kidney disease; lower right black quarter = mild left ventricular hypertrophy in the mother of the proband. Upper –: family members who were submitted to genetic testing.* B: *sequence analysis from the polymerase chain reaction product of the region with the G1644A mutation.* C: *MboI analysis (148, 124, 24 bp). Lane 1: molecular weight markers; lane 2: proband (III-2); lane 3: mother (II-2); lane 4: brother (III-1); lane 5: father (II-1); lane 6: wild type (wt) control; lane 7: uncut wt. D: secondary structure of the mitochondrial $tRNA^{Val}$. The G1644A new mutation is indicated in the* red circle. *The* black circle *indicates the other $tRNA^{Val}$ mutation identified in a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode (MELAS) syndrome[4]. HCM = hypertrophic cardiomyopathy; MWM = molecular weight markers; nd = not determined.*

Table I. Alignment of the mitochondrial transfer RNA^{Val} region including the G1644A position in 79 different species.

	Anticodon ↓	1644 ↓
Man (reference sequence)	T**TAC**ACTTAGGA	**G** ATTTCAACTTAA
Bacteria		
Methanococcus vani. (archae)	T**TAC**AAGGCGAG	**G** GTCGCCGGTTCG
Mycoplasma capricolum	T**TAC**AAGCAGGC	**G** GTCATAGGTTCA
Mycoplasma mycoides	T**TAC**AAGCAGGC	**G** GTCATAGGTTCA
Mycoplasma pneumoniae	T**TAC**AAGCATAG	**G** GTCGGGGGTTCG
Acholeplasma laidlawii	T**TAC**AAGCAGGG	**G** GTCGGCGGTTCA
Staphylococcus aureus	T**TAC**AAGCAGAG	**G** GTCGGCGGTTCG
Lactobacillus bulgaricus	T**TAC**AAGCAGAG	**G** GTCACAGGTTCG
Bacillus subtilis	T**TAC**AAGCAGAG	**G** GTCGGCGGTTCG
Bacillus sp. PS3	T**TAC**AAGCAAGG	**G** GTCGGCGGTTCG
Escherichia coli	T**TAC**AAGGAGGG	**G** GTCGGCGGTTCG
Azospirillum lipo.	T**TAC**AAGCAGAG	**G** GTCGGCGGTTCG
Unicellular eukaryotes		
Prototheca wickerhamii	T**TAC**AACGAGAT	**G** GTTAATGGTTCG
Aspergillus nidulans	T**TAC**ACACGAAG	**G** TCAGGTGTTCAA
Podospora anserina	T**TAC**ACACGGAA	**G** GCTATGAGTTCG
Saccharomyces cerevisiae	T**TAC**ACACGAAA	**G** ATTATAGGTTCG
Candida parapsilopsis	T**TAC**ACGTCTTA	**G** TTAATGGTTCGA
Torulopsis glabrata	T**TAC**ACACGAAA	**G** ATTATAGGTTCG
Trichophyton mentagrophytes	T**TAC**ACACGAAG	**G** TGCAGGTGTTCA
Animals		
Gorilla gorilla	T**TAC**ACTTAGGA	**G** ATTTCAACTTAA
Pan paniscus	T**TAC**ACTTAGGA	**G** ATTTCAACTTAA
Pan troglodytes	T**TAC**ACTTAGGA	**G** ATTTCAACTTAA
Pongo pygmaeus Abelii	T**TAC**ACTTAGGA	**G** ATTTCAATTCAA
Hylobates lar	T**TAC**ACCCGGGA	**G** ATTTCAATTAAC
Pongo pygmaeus	T**TAC**ACTTAGGA	**G** ATTTCAACTTAA
Ursus americanus	T**TAC**ACCCAGAA	**G** ATTTCACGTTAT
Talpa europea	T**TAC**ACCCAGAA	**G** ATTTCATTAAAA
Ovis aries	T**TAC**ACCTAGAA	**G** ATTTCACACATT
Hippopotamus amphibius	T**TAC**ACCCAGAA	**G** ATTTCACAATAA
Gallus gallus	T**TAC**ACCTCAAA	**G** ATACCCTCAACA
Felis catus	T**TAC**ACCCAGAA	**G** ATTTCATATTAA
Diplophos taenia	T**TAC**ACCGAAGA	**G** ACGCCCGTGCAA
Canis familiaris	C**TAC**ACCCAGAA	**G** ATTTCATTACTT
Coturnix chinensis	T**TAC**ACCTGAAA	**G** ATGCCCTTTAAA
Falco peregrinus	T**TAC**ACCTGAAA	**G** ATGTCTGTCACC
Ciconia ciconia	T**TAC**ACCTGAAA	**G** ATGTCTGCTACC
Cavia porcellus	T**TAC**ACCCGGAA	**G** ATTTCAGCCACC
Anguilla japonica	T**TAC**ACCGAGAA	**G** ACACTCGTGCAA
Equus asinus	T**TAC**ACCTAGAA	**G** ATTTCACTCAGA
Equus caballus	T**TAC**ACCTAGAA	**G** ATTTCACTCAAA
Antigonia capros	T**TAC**ACTGAGGA	**G** TCATCCGTGCAA
Rhinoceros unicornis	T**TAC**ACCCAGGA	**G** ATTTCATACAAA
Sus scrofa	T**TAC**ACCTAGAA	**G** ATCCCACAATGT
Struthio camelus	T**TAC**ACCTGAGA	**G** ATGTCTAACATA
Orycteropus afer	C**TAC**ACCCAGAA	**G** ATTTCAATAACC
Salmo salar	T**TAC**ACCGAGAA	**G** ACATCCGTGCAA
Zeus faber	T**TAC**ACCGAGAA	**G** CCGCCCGTGCAA
Ceratotherium simum	T**TAC**ACCCAGGA	**G** ATTTCATACAAA
Euthynnus alletteratus	T**TAC**ACTGAAAA	**G** TCATCCGTGCAA
Paralichtys olivaceus	T**TAC**ACCGAGGA	**G** ACATCACGTGCA
Squalus acanthias	T**TAC**ACCTGAAA	**G** ATGTCTACATAA
Ascaris suum	T**TAC**AATGAGAG	**G** GTTTATTAAGTT
Caenorhabditis elegans	T**TAC**AATGAGAG	**G** GTTTATTAAGTT
Aedes albopictus	T**TAC**ATTGAAAA	**G** AAATTTGTGCAA
Apis mellifera	T**TAC**AGTGAAAA	**G** ATTAAAAAATTA
Drosophila yakuba	T**TAC**ATTGAAAA	**G** ATTTTTGTGCAA
Pisaster ochraceus	T**TAC**ACAGAGAA	**G** AAGTTTGTGAAA
Asterina pectinifera	T**TAC**ACAGAGCT	**G** ATATTTGTGCAA
Asterias forbesii	T**TAC**ACGGAGAA	**G** AAGTTTGTGAAA
Cyprinus carpio	T**TAC**ACCGAGAA	**G** ACATCCATGCAA

(continues)

Table I. *(continued)*

	Anticodon ↓	1644 ↓
Anopheles quadrimaculatus	TTACATTGAAAA	G AAATTTGTGCAA
Crossostoma lacustre	TTACACCGAGAA	G ACATCCATGCGA
Chicken	TTACACCTGAAA	G ATACCCTCAACA
Rat	CTACACCCAGAA	G AATTCATAAAAA
Mouse	CTACACCCAGAA	G ATTTCATGACCA
Balaenoptera physalus	TTACACCTAGAA	G ATTCCACAGCCC
Balaenoptera musc.	TTACACCTAGAA	G ATTCCACAGCCC
Bovine	TTACACCTAGAA	G ACTTCATTCATT
Halichoerus grypus	TTACACCCAGAA	G ATTTCACACCCA
Phoca vitulina	TTACACCCAGAA	G ATTTCACACCCA
Aepyceros melampus	TTACACCTAGAA	G ATTTCATACACA
Boselaphus tragoc.	TTACACCTAGAA	G ATTTCACATACG
Cephalophus maxw.	TTACACCTAGAA	G ATTTCACCCACC
Damaliscus dorcas	TTACACCTAGAA	G ATTTCACACATC
Gazella thomsoni	TTACACCTAGAA	G ATTCCATACACT
Kobus ellipsiprym.	TTACACCTAGAA	G ATTTCACACGCC
Madoqua kirki	TTACACCTAGAA	G ATTTCACACACT
Oryx gazella	TTACACCTAGAA	G ATTCACATATCA
Tragelaphus imber.	TTACACCTAGAA	G ATTTCACACACC
Patient (III-2)	TTACACTTAGGA	A ATTTCAACTTAA

mtDNA mutations[28]. In our family, the mild hypertrophy of the proband's mother cannot be defined as HCM because of the borderline values of the ventricular thickness, the absence of diastolic filling abnormalities and the normal atrial size and function. Both mother and grandmother of the proband however are affected by hearing loss that constitutes one of the typical phenotypes associated with mtDNA pathological defects, while the proband's brother does not show any clinical phenotype, neither cardiac nor auditory. It is worth noting that the phenotype variability is typical of mtDNA-related diseases. However, the absence of tissue biopsies prevents any measurement of the amount of mutant DNA in different tissues and we did not expect any useful clinical information from endomyocardial biopsy that was hence not performed. The molecular genetic data in this family are those obtained from blood DNA where the amount of mutant molecules was higher in the proband than in the mother and unaffected brother (85.4, 76.0 and 74.1%, respectively). The phenotypic expression of heteroplasmic mutations is linked to the amount of mutant mtDNA, with threshold values variable in different tissues, depending on their specific energy demands. This behavior is well known and typical of mtDNA mutations[29-31];

2) the cardiac involvement: the typical HCM associated with mtDNA defects is symmetrical and frequently evolves through dilation and congestive heart failure. Our patient displayed both symmetrical hypertrophy and left ventricular dysfunction. Asymmetrical hypertrophy usually recurs in HCM caused by nuclear gene defects;

3) the ethical issue of cardiac transplantation in a patient with a complex and severe syndrome, associated with mental retardation: this aspect is beyond the scope of the present study. However, although all patients have equal rights to be given the best treatment, this particular syndrome is unlikely to enter the field of transplantation for obvious reasons. No organ transplant has ever been performed in patients with such a syndrome.

The G1644A mutation found in our family has all the necessary requirements to be defined as pathological:

• the G at the 1644 position is a very conserved base as shown by the sequence alignment reported in table I; the G at 1644 is also maintained in those mt-tRNA^{Val} where the base sequence of the region is highly variable;

• the G → A mutation at 1644 np of the tRNA^{Val} is located in the unique tRNA^{Val}, two bases 5' apart from the G1642A transition (Fig. 1D), described as being etiologic for a MELAS syndrome[4];

• the mutation, as indicated both by sequencing (Fig. 1B), agarose gel electrophoresis of the mismatch amplified mtDNA fragment (Fig. 1C) digested with *Mbo*I and densitometric analysis, is heteroplasmic;

• the mutation has not been found in our series of healthy control subjects.

In conclusion, the novel mtDNA tRNA^{Val} mutation identified in our family is associated with HCM and MELAS as well as with other minor phenotypes typical of mtDNA defects (i.e. deafness). Although only a few patients with MELAS may necessitate cardiological evaluation for a coexisting HCM (2 cases described to date[6-10,15]), we recommend that cardiologists be aware of this association as well as of the non-cardiac signs that may address the diagnosis to a mtDNA defect-related disease.

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