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SHORT REPORT

The mitochondrial DNA mutation T12297C affects a highly conserved nucleotide of tRNA$^{Leu(CUN)}$ and is associated with dilated cardiomyopathy

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Mitochondrial DNA (mtDNA) mutations have been causally linked with cardiomyopathies, both dilated (DCM) and hypertrophic. We identified the T12297C mutation in the mtDNA-tRNA$^{Leu(CUN)}$ of a 36-year-old male patient diagnosed with DCM. The mutation was heteroplasmic, with high amount (88%) of mutant DNA in the myocardium, and was absent in normal (*n*=120) and disease (*n*=150) controls. It affects a highly conserved nucleotide (adjacent to the anticodon triplet) that allows the phospho-ribose backbone to turn and form the loop. The potential pathological role of T12297C mutation is further supported by its recent identification in another unrelated Italian family with DCM associated with endocardial fibroelastosis. In the variable loop of the same tRNA, our patient also carried the A12308G transition that is debated as pathological mutation or neutral polymorphism in progressive external ophthalmoplegia: the two defects could exert a synergistic effect on the tRNA structure and function. The endomyocardial biopsy study showed abnormal ring-like mitochondria and occasional cytochrome *c* oxydase negative myocytes. Overall, the heteroplasmy, the highly conserved position of the mutated nucleotide, the absence of the mutation in large series of diseased and normal controls, and the cardiac mitochondrial changes support a causative link of the mutation with the disease. *European Journal of Human Genetics* (2001) **9,** 311 – 315.

Keywords: dilated cardiomyopathy; mitochondrial DNA; tRNA$^{Leu(CUN)}$

Introduction

Several mitochondrial DNA (mtDNA) mutations have been causally linked both with multiorgan syndromes[1–6] and isolated cardiomyopathies, either hypertrophic (HCM)[7–10] or dilated (DCM).[11–13]

Given that isolated DCMs associated with mtDNA defects may look clinically alike to non-mitochondrial cardiomyopathies, the ultrastructural mitochondrial morphology and/or the mitochondrial histoenzymatic study of the endomyocardial biopsies (EMB) may usefully guide molecular analysis.[4,7,8,14,15]

In the present study we report a mtDNA point mutation, T12297C, in the tRNA$^{Leu(CUN)}$, heteroplasmic, absent from normal controls, affecting a highly conserved region of the mtDNA, in a male patient diagnosed with DCM. The mutation has been reported in December 1999 in another Italian family (unrelated to our patient) with cardiomyopathy and endocardial fibroelastosis.[16]

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Materials and methods

Case description

A 36-year-old male was referred to our centre for congestive heart failure dating back 1 month. The echocardiographic study showed increased ventricular diameters (diastolic:

78 mm, systolic: 55 mm) and systolic dysfunction (EF: 30%); coronary arteries were angiographically normal. Biochemical assays and Creatin Kinase (CK) MM and MB isoforms were within normal ranges. The patient underwent endomyocardial biopsy (EMB). The conventional light microscopy study of EMB sections stained with Hematoxylin-Eosin and Movat pentachrome was uninformative, and showed irregular size and shape of the myocytes and nuclei, and interstitial fibrosis. Modified Gomori showed abundant fucsinophilic material but most myocytes had hypercontraction artifactious bands, which limited the understanding of this finding. Cytochrome *c* oxydase (COX) and NADH reactions documented irregular intensity, with few COX-negative cells (Figure 1a). Succinic dehydrogenase reaction looked normal.

The ultrastructural study showed mitochondrial proliferation, with a wide range of changes in size and shape, and cristolysis. In several cells, we found mitochondria exclusively constituted of peripheral rings of mitochondrial membranes, devoid of internal cristae (Figure 2). This pattern, never observed in other cardiomyopathies (from 1984 to 1999 the ultrastructural study has been routinely done in 1244 EMB) was considered a useful marker for progressing with mtDNA analysis.

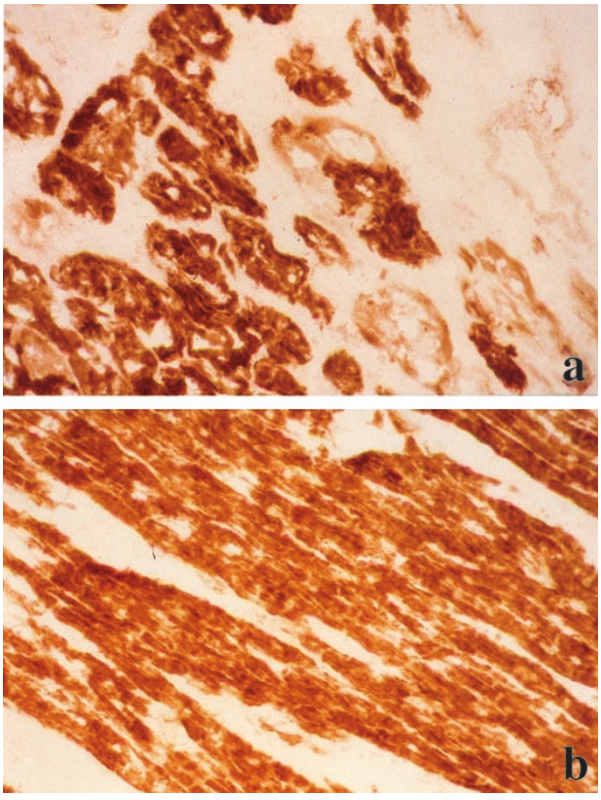

Figure 1 Cytochrome *c* oxidase on the endomyocardial biopsy of the patient **a** and in a normal donor heart biopsy **b**. (COX, **a**, ×180 and **b**, ×120).

Other ultrastructural changes consisted of myocyte damage with myofibrillar loss, lipid droplets, and interstitial fibrosis.

Molecular analysis

Total genomic DNA was purified from EMB and peripheral blood with conventional methods;[17] mtDNA was analysed as previously described.[10,13] MtDNA regions (encompassing the entire genome) were amplified by PCR from 0.1 μg of the total DNA, and subjected to sequence analysis using a 377 and 373 sequencer (ABI-Perkin Elmer) under conditions recommended by the manufacturer. The mutation found in the tRNA$^{Leu(CUN)}$ introduces a *Ava*II restriction site that was used to assess the heteroplasmy. The relative amounts of the mutant mitochondrial genomes were quantitated with a Bioprofile Bio-1D blot analyser (Vilber Lourmat Biotechnology Division, Marne La Vallee, France).

Results

We identified the T12297C mutation in the tRNA$^{Leu(CUN)}$ of the mtDNA (Figure 3a). In the same tRNA we found the A12308G homoplasmic transition. Further polymorphic changes in the mtDNA were: A750G in 12sRNA, A14438G in 12sRNA, A1811G in 16sRNA, A2706G in 16sRNA, G12372A in ND5 (CTG->CTA; Leu->Leu) and C14620T in ND6 (CCC->TCC; Pro->Ser).

The T12297C mutation, which affects a highly conserved nucleotide (Table 1), was heteroplasmic, with 70% mutant in peripheral blood DNA and 88% mutant in the affected heart DNA (Figure 3b). After detection of the T12297C mutation, we informed the patient according to the rules of our familial DCM program. We invited the patient to extend the

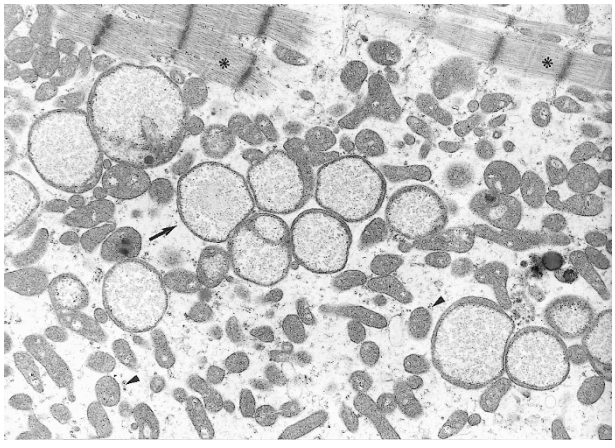

Figure 2 Electron micrograph showing the mitochondrial changes in the endomyocardial biopsy of the patient with mtDNA mutation T12297C: there is a wide variation in size and shape and several mitochondria are constituted of a peripheral ring of cristae. (Uranile acetate, lead citrate). →: ring-like mitochondria; ▶: normal-sized mitochondria; *: myofibrils.

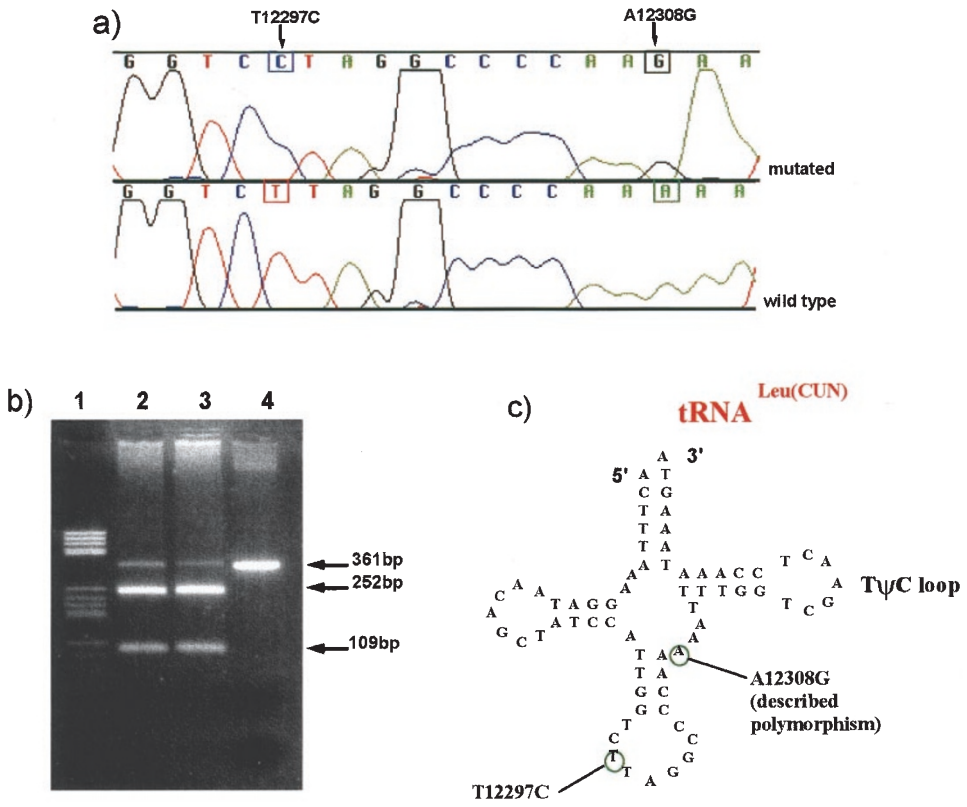

Figure 3 **a**, Sequence analysis from PCR product of the region with the T12297C mutation and the A12308G polymorphism. **b**, *Ava*II RFLP analysis (361 bp, 252 bp, 109 bp). Lane 1: molecular weight markers, lane 2: blood-DNA, lane 3: heart DNA, lane 4: normal control DNA. **c**, Secondary structure of the mitochondrial tRNA$^{leu(CUN)}$.

information to his maternal relatives and suggested the possibility of a genetic counselling, with clinical (non-invasive) and molecular screening for all consenting relatives. Although in most cases the patients collaborate and accept the screening program, this patient refused our proposal. Therefore the possible maternal inheritance of the disease remains unanswered.

Discussion

The heteroplasmic T12297C mutation in the tRNA$^{Leu(CUN)}$ of the mtDNA found in our patient is likely to be causally linked with the disease. The mutation affects a conserved nucleotide position adjacent to the anticodon triplet. This nucleotide plays a key role in mRNA-anticodon interactions, allowing the phospho-ribose backbone to turn and form the loop (U-turn) (Figure 3c). The high level of mutant DNA in the cardiac tissue highlights the potential role of this mutation in the cardiomyopathy. Finally, the same mutation was absent in more than 150 patients with DCM and 120 controls.

Further support to the pathological significance of this mutation comes from its recent identification in another unrelated Italian family with cardiomyopathy.[16] The clinical phenotype in our patient slightly differs from that reported by Tessa *et al*: typical DCM (present case) and DCM plus endocardial fibroelastosis,[16] respectively. The phenotypical heterogeneity typically recurs in mtDNA-related disorders. For sure, our proband and Tessa *et al* family are unrelated, given the identification, in our patient, of the A12308G transition in the same tRNA, as well as of other polymorphisms, not reported in the other family.[16] A possible synergistic effect of T12297C and A12308G can not be excluded.

The mitochondrial origin of a cardiomyopathy is unlikely to be identified, unless it is specifically investigated. Patients with exclusive cardiac involvement, with negative or unavailable family history, without clinical evidence of concomitant myopathy, and serum CK-MM within normal ranges, are unlikely to be suspected by the clinical cardiologists as affected by mitochondrial disease. In this clinical cardiology setting, the only practical guide addressing to a possible mitochondrial origin of the cardiomyopathy is the endomyocardial biopsy, with ultrastructural and mitochondrial enzyme studies. EMB samples must be used to exclude the inflammatory or dystrophic[18] or autoimmune, etc. origin of the disease. Therefore, any morphological change addressing to a possible aetiology of the cardiomyopathy must be taken into account.

The informativeness of EMB is minor to that of muscle biopsy due to the small sample size and the low sample number, as well as for the procedure-related contraction bands affecting myocardial biopsies. In particular, the

Table 1 A~lignment of the anticodon region, underlined, of mt-tRNA$^{leu\ (CUN)}$ with the adjacent highly conserved 5′ T at 12297 nt in 60 different species. The polymorphic mutation at 12308 nt is also indicated

PHAGE T5	GACAATACGGCC**T**TAGATTCCGT**A**-GCT--TAAA--TGCG-T
METHANOCOC.VANI.	AAGATGCAGGAC**T**TAGAATCCTG**T**-CCA--GTAG--TGGTTC
METHANOTHERM. FER.	AAGGGGACAGGC**T**TAGGACCTGT**T**-GGC--GTTAG-GCTT-C
MYCOPLASMA CAPRIC.	GACGCACTAGAC**T**TAGGATCTAG**C**-GTC--TTT---GACG-T
MYCOPLASMA PNEUMO.	GACGCGCTAGAC**T**TAGGATCTAG**T**-TTCA-TTG--TGGAG-T
MYCOPLASMA PG50	GACGCACTAGAC**T**TAGGATCTAG**C**-GTC--TTT---GACG-T
ACHOLEPLASMA LAID.	GACGCACTAGAC**T**TAGGATCTAG**C**-GCT--TTAC--GGCA-T
STAPHYLOCOC. AURE.	GACGCACTAGAC**T**TAGGATCTAG**C**-GCC--TTTAC-GGCG-T
BACILLUS SUBTILIS	GACGCGCTAGAC**T**TAGGATCTAG**T**-GTC--TTTAT-GACG-T
E. COLI	GACGCACCAGAT**T**TAGGTTCTGG**C**-GCC--GCAA--GGTG-T
PHOTOBAC. LEIOGNA.	GACGCACTAGAT**T**TAGGTTCATG**C**-GCCT-GTA--AGGTG-T
LYCOPERSICON ESCU.	GACACGCTGCTC**T**TAGGAAGCAG**T**-GC---TAAT---GCA-T
EUGLENA GRACILIS	GACGCGCAGGAT**T**TAGGTTCCTG**T**-GTC--TTTAT-GATG-T
MARCHANTIA POLYM.	GACACGCTGCTC**T**TAGGAAGCAG**T**-GC---TAAG---GCT-T
ORYZA SATIVA	GACACGCTGCTC**T**TAGGAAGCAG**T**-GCT--CA----AGCA-T
EPIFAGUS VIRGINIA.	GACACGTTGCTC**T**TAGGAAGCAG**T**-GCT--AT----AGCA-T
NICOTIANA TABACUM	GACACGCTGCTC**T**TAGGAAGCAG**T**-GC---TAAT---GCA-T
PINUS THUNBERGII	GACACGCTGCTC**T**TAGGAAGCAG**T**-GC---GAGA---GCA-T
PROTOTHECA WICKER.	TACACACCAGGT**T**TAGGTTCTGG**A**-GATT-AAG--AGTCTTT
ASPERGILLUS NIDUL.	AACAGGTTCCGC**T**TAGGACGGAA**T**-AGT--CAA---ACTT-T
PICHIA PIJPERI	GACGCGATTAGT**T**TAGGTCTAAT**T**-TATT-TTT--AATAA-T
MARCHANTIA POLYM.	GACATGCCAGGT**T**TAGGTTCTGG**T**-GACCATAA-TGTTCG-T
ASCARIS SUUM	AATATGATAGAT**T**TAGGTTCTAT**A**G--------------A-T
CAENORHABDI. ELEG.	AATACATTAGAT**T**TAGGTTCTAA**A**G--------------A-T
MYTILUS EDULIS	AATGCGCTTGAT**T**TAGGATCAAT**T**T--------------A-T
LOCUSTA MIGRATORIA	AGTGCAATGAAT**T**TAGAATTCAT**T**T--------------A-T
AEDES ALBOPICTUS	AGTGCAGTAAAT**T**TAGAATTTAT**T**T--------------A-T
APIS MELLIFERA	AGTGCATTAAAT**T**TAGGATTTAA**T**T--------------TAT
DROSOPHILA YAKUBA	AGTGCAATAAAT**T**TAGAATTTAT**A**T--------------A-T
PISASTER OCHRACEUS	AATGCTTGAGAT**T**TAGGTTCTTA**C**A--------------T-C
ASTERINA PECTINI.	ACTGCAGTAGAT**T**TAGGATCTAC**G**A--------------T-C
ASTERIAS FORBESII	AATGCTTGAGAT**T**TAGGTTCTCA**C**A--------------T-C
CYPRINUS CARPIO	CATCCGTTGGTC**T**TAGGAACCAA**A**A--------------ACT
PARACENTROTUS LIV.	AATGCAAAAGAC**T**TAGGATTTTT**C**A--------------T-C
ANOPHELES QUADRIM.	AGTGCAATAAAT**T**TAGAATTTAT**A**A--------------A-T
STRONGYLOCEN.PURP.	TATGCAAAAGAC**T**TAGGATCTTT**T**A--------------A-C
GADUS MORHUA	CATCCGTTGGTC**T**TAGGAACCAA**A**A--------------ACT
ACANTHAMOEBA CAST.	GACAGGCTGATT**T**TAGAAATCAG**T**-GGG--TAAT--TCCG-T
XENOPUS LAEVIS	TATCCGCTGGTC**T**TAGGAACCAG**A**A--------------ACT
RANA CATESBEIANA	CCTCCACTGGTC**T**TAGGCGCCAG**C**A--------------TCT
CROSSOSTOMA LACUS.	CATCCATTGGTC**T**TAGGAACCAA**A**A--------------ACT
CHICKEN	AATCCGTTGGTC**T**TAGGAACCAC**C**T--------------A-T
RAT	AATCCATTGGTC**T**TAGGAACCAA**A**A--------------A-C
MOUSE	AATCCATTGGTC**T**TAGGAACCAA**A**A--------------A-C
BALAENOPTERA PHYS.	TATCCATTGGTC**T**TAGGAGCCAA**A**A----------------A
BALAENOPTERA MUSC.	TATCCATTGGTC**T**TAGGAACCAA**A**A----------------A
BOVINE	TATCCGTTGGTC**T**TAGGAACCAA**A**A----------------A
PHOCA VITULINA	AATCCATTGGTC**T**TAGGAACCAA**A**A----------------A
MACACA FUSCATA	TATCCATTGACC**T**TAGGAGTCAA**A**A--------------A-C
MACACA MULATTA	TATCCATTGACC**T**TAGGAGTCAA**A**A--------------A-T
MACACA FASCICULA.	TATCCATTGACC**T**TAGGAGTCAA**A**A--------------A-C
MACACA SYLVANUS	TATCCATTGGCC**T**TAGGAGTCAA**A**A--------------A-T
SAIMIRI SCIUREUS	TATCCATTGGTC**T**TAGGAGCCAA**A**A--------------A-C
TARSIUS SYRICHTA	AATCCATCGGTC**T**TAGGAACCGA**A**A----------------A
LEMUR CATTA	AATCCATTGGCC**T**TAGGAGCCAA**A**A----------------A
CHIMPANZEE	CATCCGTTGGTC**T**TAGGCCCCAA**A**A--------------A-T
GIBBON	TATCCATTGGTC**T**TAGGACCCAA**A**A--------------A-T
GORILLA	TATCCATTGGTC**T**TAGGACCCAA**A**A--------------A-T
ORANG UTAN	TATCCCTTGGTC**T**TAGGATCCAA**A**A--------------A-T
HUMAN	TATCCATTGGTC**T**TAGGCCCCAA**A**A--------------A-T
PATIENT	TATCCATTGGTC**C**TAGGCCCCAA**G**A--------------A-T
	12297 12308

benefits deriving from the histoenzymatic study of the EMB samples are severely limited by the artifactious contraction bands, while the ultrastructural study retains its informativeness, especially for organelles. Therefore, both histoenzymatic and mitochondrial ultrastructural changes should be used to guide molecular analysis of the mtDNA.

Once identified, a pathologic DNA change associated with DCM could contribute to clarify the pathogenetic profile of the disease, and could offer chances for pre-clinical diagnosis, or for genotype-phenotype correlation studies. This information could alert clinicians to monitor family members who carry the mutation, thus preventing life-threatening ventricular arrhythmias. A major limit for cardiomyopathies associated with mtDNA defects is that the segregation of a mutation with the phenotype is highly dependent on the threshold value of the given mutation in affected tissues rather than on the mutation itself. This makes difficult the definition of the causative link between mtDNA defects and phenotypes because maternal relatives, with and without phenotype, carry the mutation. The useful information derives from measuring the amount of mutant DNA in the myocardium. This can be done for patients who undergo EMB but not in healthy family members. For these latter, the mutation, as well as its heteroplasmic condition, can be tested only in peripheral blood DNA and therefore do not inform on the amount of mutated DNA in the myocardial tissue. In our patient, the mutant DNA measured in peripheral blood was 70%, similar to that (65%) found in the healthy mother of the two patients described by Tessa *et al*.[16]

In conclusion, our overall findings, although documented only in the index patient, support the association of the T12297C mutation with the myocardial disease and fits with the clinical heterogeneity that characterises the clinical phenotypes related to mtDNA defects.

Acknowledgements

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