SHORT REPORT

# Antenatal mitochondrial disease caused by mitochondrial ribosomal protein (*MRPS22*) mutation

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*J Med Genet* 2007;**44**:784–786. doi: 10.1136/jmg.2007.053116

Three patients born to the same set of consanguineous parents presented with antenatal skin oedema, hypotonia, cardiomyopathy and tubulopathy. The enzymatic activities of multiple mitochondrial respiratory chain complexes were reduced in muscle. Marked reduction of 12s rRNA, the core of the mitochondrial small ribosomal subunit, was found in fibroblasts. Homozygosity mapping led to the identification of a mutation in the *MRPS22* gene, which encodes a mitochondrial ribosomal protein. Transfection of the patient cells with wild-type *MRPS22* cDNA increased the 12s rRNA content and normalised the enzymatic activities. Quantification of mitochondrial transcripts is advisable in patients with multiple defects of the mitochondrial respiratory chain.

Of the 85 mitochondrial respiratory chain subunits, 13 are synthesised within the organelle by the mitochondrial ribosome. This translation apparatus consists of two ribosomal RNA (rRNA) transcripts encoded by the mitochondrial genome, and nearly 80 nuclear-encoded ribosomal proteins. In addition to the 22 mitochondrial-encoded transfer RNAs (tRNAs), mitochondrial translation requires a large set of imported proteins, including translation factors, aminoacyl-tRNA synthetases and tRNA modification enzymes. Defects in nuclear-encoded components of the mitochondrial protein synthesis machinery are found in 5–10% of infants referred for enzymatic investigations of the oxidative phosphorylation system in muscle.[1 2] We now describe a new defect in the mitochondrial ribosome, present before birth.

Two female babies, born at term to consanguineous parents (first cousins), presented within hours of birth with severe muscle hypotonia, marked lactic acidaemia and hyperammonaemia. The pregnancies were uneventful, but at around 36 weeks of gestation, fetal sonography revealed generalised oedema, especially at the neck, labia, palms and feet. At birth, there was prominent subcutaneous oedema and ascites (fig 1). Hypertrophic cardiomyopathy and tubulopathy developed in the first week and the patients died at 2–22 days of age.

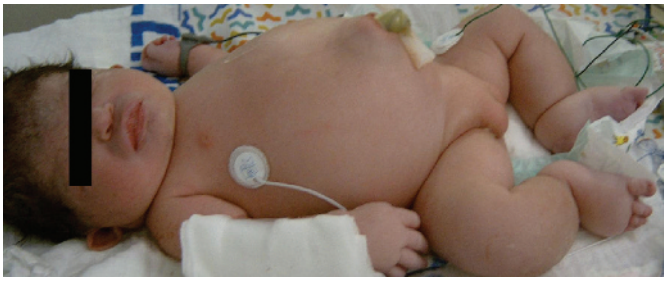

**Figure 1** Patient 3 at 4 hours of age. Parental/guardian informed consent was obtained for publication of this figure.

In the muscle mitochondria of the first affected infant, the enzymatic activities of the respiratory chain complexes I, III, IV (COX) and V were reduced to 8–30% of the control mean. The activity of complex II, the only complex that is encoded solely by the nuclear genome, was normal. A muscle biopsy from the second patient was denied, but COX activity in lymphocytes was reduced to 47–62%. Based on the enzymatic findings, a defect in the synthesis of the mitochondrial-encoded proteins was assumed. Initially, Southern blot analysis of muscle DNA revealed normal sized mtDNA but decreased mtDNA content, suggesting mtDNA depletion.[3] A similarly affected daughter was recently born, enabling homozygosity mapping analysis in the family, concomitant with revision of the molecular studies. Re-evaluating the mtDNA content by means of real-time quantitative PCR (qPCR) with an expanded age-matched control range, found a normal mtDNA:nuclear DNA ratio in the muscle of the first patient.

To investigate whether a defect in mitochondrial transcription is responsible for the multiple enzymatic defects, three mitochondrial transcripts, COX2 mRNA, 12s rRNA and 16s rRNA, were quantified in the patients' fibroblasts using real-time qPCR. The analysis revealed a striking reduction of 12s rRNA content to 10% of the control mean and a moderate reduction of 16s rRNA and COX2 mRNA to 41% and 45% of the control mean, respectively. Notably, the sequence of the 12s rRNA was similar to the consensus sequence (http://www.mitomap.org/).

Bacterial rRNAs are stable only if incorporated into the ribosomal subunits;[4] we therefore speculate that the markedly reduced 12S rRNA level was the result of a defect in one of the 29 proteins that, together with the 12s rRNA, constitute the small subunit of the mitochondrial ribosome.[5]

The search for homozygous regions using the Affymetrix Human Mapping 50K Array Xba240 (Affymetrix, Santa Clara, California, USA) in DNA samples of the affected patients, performed as described previously,[6] revealed a single, large genomic homozygous region on chromosome 3 at 118.723–174.963 Mb (marker rs1381801–rs1352416). The result was verified by genotyping a set of polymorphic microsatellite markers that spanned the region (D3S1267, D3S1292, D3S1569, D3S1279, D3S1594, D3S1264 and D3S1614). The three patients were homozygous for all the markers, whereas the parents and their healthy daughter were heterozygous for the same allele. This large region encompasses 442 open-reading frames, of which 7 were annotated as mitochondrial, including MRPL3, mitochondrial elongation factor G1 (which were both sequenced and found to be normal), and *MRPS22*. The latter gene encodes a protein of 319 amino acids. Sequence determination of its cDNA revealed a G→A substitution at position 509, which is predicted to replace a highly conserved arginine in codon 170 by histidine (fig 2A–D). The three patients were homozygous, and the parents and their healthy

**Abbreviations:** qPCR, quantitative PCR; tRNA, transfer RNA

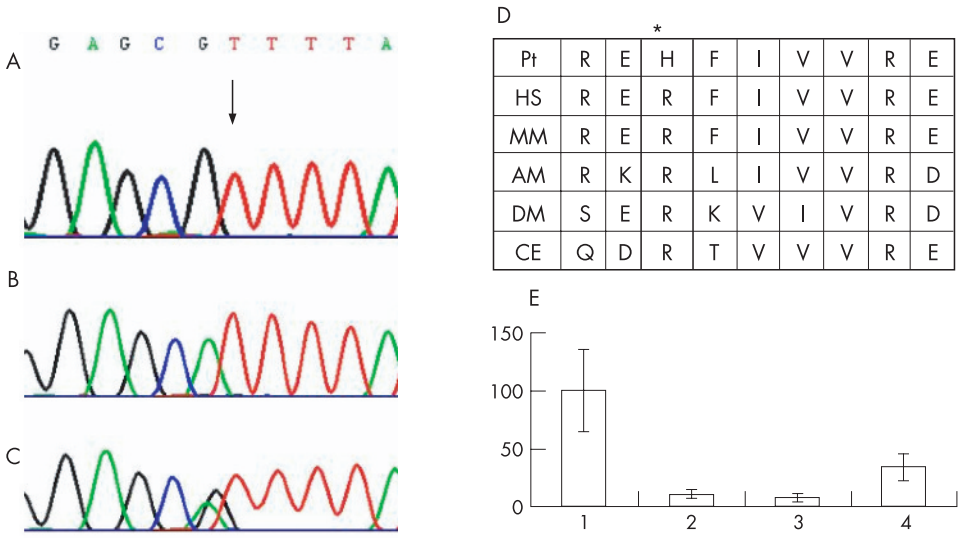


**Figure 2** *MRPS22* mutation, conservation of the mutated residue and restoration of the 12s rRNA level in patient cells. (A) *MRPS22* DNA sequence analysis of the patient showing a G nucleotide at position 509 (arrow). Note an A nucleotide at this position in (B) a healthy control and an A/G in (C) the mother's sequence, disclosing a carrier state. (D) The mutation affects a highly conserved arginine at codon 170 (marked by an asterisk) in the amino acid sequence of the patient (Pt) amino acid. Interspecies comparison of the amino acids bordering the mutation between human (HS), mouse (MM), honeybee (AM; *Apis mellifera*), fruitfly (DM; *Drosophila melanogaster*) and the worm *Caenorhabditis elegans* (CE) (protein C14A4.2), shows the conservation. (E) The 12s rRNA transcript level in: bar 1, control cells; bar 2, patient cells; bar 3, patient cells transfected with an empty lentiviral construct; bar 4, patient cells transfected with a construct containing the wild-type *MRPS22* cDNA. Results are expressed as a percentage of the control mean.

daughter were heterozygous for the mutation, which was not present in 52 ethnically matched controls. Because the *MRPS22* protein has no counterpart in the *Escherichia coli* ribosome, and the functional domains are not recognised, the pathogenic potential of this mutation was unclear.

To address this question, the normal *MRPS22* cDNA was cloned into a pLenti6/V5-D-TOPO 6969-bp expression vector by directional TOPO cloning (Invitrogen Corp., Carlsbad, California, USA). The recombinant vector was propagated in 293FT cells according to the manufacturer's instructions (ViraPower; Invitrogen). The patient cells were infected with the recombinant virus, and stably transduced cells were selected by blasticin. The 12s rRNA content in patient cells infected with the *MRPS22* construct was 34 (4)% of the control mean, whereas in cells infected with an empty vector, the 12s rRNA content was 10 (4)% (fig 2E). The enzymatic activity of COX concomitantly increased from 27–33% to 77–92% of the control mean. Both 12S rRNA content and COX activity remained unchanged in patient cells that were transfected with the empty viral construct. These experiments established the pathogenic role of the mutated *MRPS22* protein.

Defects in nuclear-encoded components of the translation system comprise a novel group of mitochondrial respiratory chain defects. The patients may appear normal at birth[7 8] but may also display intrauterine growth retardation, dysmorphic signs and cerebral malformations.[8 9] It is of interest that the first reported patient with a defect in the mitochondrial ribosome was also born with subcutaneous oedema.[1]

The *MRPS22* gene is the second ribosomal protein involved in mitochondrial disease in humans, and this is the first report of a mutation in this gene. The study underlines the superiority of real-time qPCR over Southern blot for mtDNA quantification; the former requires a smaller amount of tissue and is far more accurate. Our findings also highlight the usefulness of mitochondrial transcript quantification, which directed us to a single gene within a very large chromosomal region.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the collaboration of the patients' family, Dr Mira Korner of the National Center of Genomic Technologies, at the Hebrew University, Jerusalem, and Corinne Belaiche for OXPHOS analysis. This work was supported in part by the Association Française contre les Myopathies (AFM), by the Israel Science Foundation (grant 1354–2005), France–Israel Ministries of Science (grant no 3-904) and by the US–Israel Binational Science Foundation (grant 032-2005).

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Competing interests: none declared.

Parental/guardian informed consent was obtained for publication of fig 1.

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Received 16 July 2007
Revised 28 July 2007
Accepted 21 August 2007
**Published Online First 7 September 2007**

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