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CASE REPORT

COXPD9 an Evolving Multisystem Disease; Congenital Lactic Acidosis, Sensorineural Hearing Loss, Hypertrophic Cardiomyopathy, Cirrhosis and Interstitial Nephritis

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Abstract We present the second report of combined oxidative phosphorylation deficiency-9. The infant presented in the neonatal period with poor feeding, lactic acidosis and sensorineural hearing loss. He subsequently developed a lethal hypertrophic cardiomyopathy during infancy. Cirrhosis and interstitial nephritis were identified at autopsy. Exome sequencing has detected compound heterozygous mutations in the *MRPL3* gene which encodes a large mitochondrial ribosome subunit protein. We identified a known heterozygous variant NM_007208 c.950>G (Pro317Arg) in the *MRPL3* gene and a novel heterozygous mutation NM_007208 c.49delC p.(Arg17Aspfs*57). Mutations in *MRPL3* have previously been shown to alter ribosome assembly and cause abnormal function of multiple respiratory chain complexes. Our case adds to the evolving knowledge of disorders of mitochondrial translation.

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Introduction

Mitochondrial disorders comprise a clinically and genetically diverse group of diseases affecting cellular energy production through mitochondrial oxidative phosphorylation. Mutations in mitochondrial DNA and nuclear genes encoding the proteins which comprise the five complexes of the mitochondrial respiratory chain are well characterised. The mechanisms and disorders of mitochondrial DNA maintenance, replication and transcription are less well understood. Mitochondrial translation involves greater than one hundred proteins which are nuclear gene encoded (Pearce et al. 2013; Ottl et al. 2016).

The mitochondrial ribosome (mitoribosome) synthesises 13 components of the mitochondrial oxidative phosphorylation complex and consists of over 80 interconnected proteins. Mitoribosomes are composed of a large (39S) subunit containing a 16S ribosomal RNA (rRNA) and a small (28S) subunit containing a 12S rRNA. The small subunit contains approximately 30 proteins and the large subunit contains approximately 50 proteins (Pearce et al. 2013).

To date, pathogenic mutations in only six genes encoding mitochondrial ribosomal proteins have been reported in association with clinical phenotypes (see Table 1), i.e. *MRPL3* gene (MIM 607118, phenotype combined

Table 1 Mitochondrial ribosomal disorders: major features

Gene	*MRPL3*	*MRPS7*	*MRPS16*	*MRPS22*	*MRPL44*	*MRPL12*
Sensorineural hearing loss	1/5	2/2	0/1	0/5	0/4	0/1
Lactic acidosis	5/5	2/2	1/1	5/5	3/4	1/1
Structural brain anomalies	2/5 cortical hypergyria	0/2	Corpus callosal agenesis, ventricular dilatation	2/5 corpus callosum and other abnormalities (3 not imaged)	1/4	1/1
Cardiomyopathy	5/5	0/2	0/1	4/5	4/4	0/1
Hepatic involvement	4/5	1/2	1/1	1/5 (multiple organ dysfunction)	4/4	1/1 (hepatomegaly)
Renal involvement	1/5	2/2	0/1	4/5 (tubulopathy, multiple organ dysfunction)	1/4	0/1
Developmental delay	2/5	0/2	Neonatal demise	1/1 surviving the neonatal period	1/4	1/1
Lethality	3/5	2/2	1/1	4/5	1/4	1/1
Reference	3 and current case	9	7	8,10,11	5,6	4

oxidative phosphorylation deficiency-9 (COXPD9 MIM 614582) (Galmiche et al. 2011), *MRPL12* (MIM602375) (Serre et al. 2013), *MRPL44* (MIM641849, phenotype COXPD16 MIM615395) (Carroll et al. 2013; Distelmaier et al. 2015), *MRPS16* (MIM 610498) (Miller et al. 2004), *MRPS22* (MIM605810; phenotype COXPD5 MIM 611719) (Smits et al. 2011; Baertling et al. 2015) and *MRPS7* (MIM 611974)) (Menezes et al. 2015). Pathogenic mutations in *MRPL3* are responsible for the clinical phenotype of COXPD9 in which severe hypertrophic cardiomyopathy is a central presentation. Our patient expands the phenotype of COXPD9 to also include neonatal lactic acidosis, sensorineural hearing loss (SNHL), cirrhosis and interstitial nephritis.

Case Report

Our patient is a male infant born at 39 weeks' gestation via elective lower uterine segment caesarean section. Apgar scores were 9 at 1 and 5 min post birth. His initial physical exam was unremarkable and he was transferred to the postnatal ward. Over the ensuing 24 h, he was noted to be lethargic and feed poorly and hypoglycaemic via glucometer testing. Formal bloods demonstrated a true blood glucose of 1.4 mmol/L and a metabolic acidosis with a bicarbonate level of 7 mmol/L and an elevated anion gap of 28. Subsequent routine bloods revealed mildly deranged transaminases which peaked on day 4 (gamma-glutamyl transferase GGT 280 U/L (0–70), alanine aminotransferase ALT 164 U/L (0–45), aspartate aminotransferase AST 941 U/L (0–41)), lactate of 10.7 mmol/L, blood pyruvate 563 umol/L and an elevated lactate/pyruvate ratio of 19. Creatine kinase was mildly elevated at 580 U/L (0–190). A urine metabolic screen (organic acids and amino acids) demonstrated lactic acid, ketoacidosis and mildly elevated pyruvate and fumarate, all suggestive of mitochondrial dysfunction. Magnetic resonance imaging (MRI) scan in the neonatal period revealed a structurally normal brain, and magnetic resonance spectroscopy (MRS) demonstrated a lactate peak in the basal ganglia. The infant was commenced on a trial of thiamine and co-enzyme Q10 supplementation which reduced the lactic acidosis from a peak of 16.1 mmol/L pre-therapy to a range of 4–5 mmol/L within 1 week post-therapy.

The patient failed newborn hearing screening in both ears and was referred for formal audiological assessments. Physiological testing (optoacoustic emissions, auditory brain stem response, auditory steady-state response) confirmed a severe sloping bilateral SNHL, and he was fitted with bilateral Naida Q 70SO hearing aids at 1 month of age. At 7 months of age, he was reassessed as part of the cochlear implant candidacy programme. Tympanography demonstrated normal middle ear pressures and compliance. Physiological testing with and without bone conductors again confirmed a severe sloping audiogram in keeping with profound SNHL. Bilateral cochlear implants were inserted at 9 months of age without incident. At this point in time, he was progressing normally from a developmental perspective although his growth was suboptimal.

Echocardiography in the newborn period showed a structurally normal heart with a patent foramen ovale and normal left ventricular function. Follow-up echocardiography at 8 months of age revealed hypertrophic cardiomyopathy, with the myocardial thickness having increased from 3 mm in the newborn period to 7 mm (z-score = +2.6).

Systolic function remained normal and there was no left ventricular outflow tract obstruction.

At the age of 11 months, the patient developed bronchiolitis and rapidly deteriorated, with hypotension, hypoglycaemia and severe metabolic acidosis. Cardiac arrest ensued and he was unable to be resuscitated. An autopsy was conducted, with the cause of death thought to be cardiac decompensation due to an unidentified respiratory virus. Microscopic examination of the liver revealed mild periportal steatosis and mild lymphoplasmacytic infiltration with minimal fibrosis. A chronic inflammatory cell infiltrate surrounded the renal tubules in keeping with evolving interstitial nephritis. The glomeruli were normally formed. Cardiac histology demonstrated cardiac muscle fibre disarray and cytomegaly in keeping with hypertrophic cardiomyopathy. Myocardial thickness had increased to 15 mm.

Genetic testing for common mitochondrial DNA point mutations and deletions including the three common *POLG* mutations (MIM174763) was negative. Subsequently next-generation sequencing was performed at the Australian Genome Research Facility. After enrichment of all the coding and flanking intronic regions, sequencing analysis was performed using an Illumina HiSeq platform. Quality criteria required at least 10 reads per base; however, 97.7% of targeted regions achieved ×100 coverage and 99.7% achieved ×10 coverage. Only sequence variations with an allele frequency <1% were considered pathogenic. The following *MRPL3* sequence variants were identified, NM_007208 c.950C>G (Pro317Arg) and NM_007208 c.49delC p.(Arg17Aspfs*57). These mutations were confirmed by Sanger sequencing. The patient's father was found to be heterozygous NM_007208 c.950C>G (Pro317Arg) and his mother heterozygous NM_007208 c.49delC p.(Arg17Aspfs*57). No other candidate genes were identified. There were no sequence variants identified in genes known to be associated with SNHL.

The *MRPL3* NM_007208 c.950C>G p.(Pro317Arg) variant is located on chromosome 3 at position 131,181,664 bp. It overlaps the coding sequence of at least one transcript of gene *MRPL3*. The reference allele for this variant is G, whereas the alternative allele is C. This variant is predicted to be a missense mutation which alters the protein's amino acid from proline (Pro) to arginine (Arg). The prediction for p.(Pro344Arg)/p.(Pro86Arg)/p.(Pro212Arg)/p.(Pro317Arg) is based on four annotated transcripts for that gene locus. The BLOSUM62 substitution matrix reports a score of −2 for this alteration. The mutation has been described in patients with COXPD9 deficiency (Galmiche et al. 2011) and classified as pathogenic (ID RCV000023618). There was no alternative allele frequency listed for this variant in the 1,000 genomes data set. There was no minor allele frequency listed for this variant in the NHLBI GO Exome Sequencing Project (ESP6500) data set. This variant overlaps with evolutionary constrained element (detected using SiPhy-ω and SiPhy-π statistics). The conservation across 28 species is described with PhyloP (score: 2.44). GERP identifies constrained elements in multiple alignments by quantifying substitution deficits (score: 5.26).

The *MRPL3* NM_007208 c.49delC p.(Arg17Aspfs*57) heterozygous deletion is located on chromosome 3 at position 131,221,616 bp. It overlaps the intronic region of gene *MRPL3*. It overlaps the coding sequence of at least one transcript of gene *MRPL3*. The reference allele for this variant is CG, whereas the alternative allele is C. This variant is predicted to be a nonsense mutation which alters the protein's amino acid sequence and leads to a premature stop codon. The prediction for p.(Arg17Aspfs*57) is based on one annotated transcript for that gene locus. This variant is predicted to be a nonsense mutation which alters the protein's amino acid sequence and leads to a premature stop codon. The variant was not previously reported in dbSNP. There has been no clinical classification available for this novel variant. There was no alternative allele frequency listed for this variant in the 1,000 genomes data set. There was no minor allele frequency listed for this variant in the NHLBI GO Exome Sequencing Project (ESP6500) data set. The amino acid substitution is predicted to be damaging (SIFT score: −1.00). The accuracy of these tools is unknown. Variant overlaps with evolutionary constrained element (detected using SiPhy-ω and SiPhy-π statistics). The conservation across 28 species is described with PhyloP (score: 2.31). GERP identifies constrained elements in multiple alignments by quantifying substitution deficits (score: 3.61).

Discussion

This is only the second report of an individual with COXPD9 due to pathogenic *MRPL3* mutations. The previous cases were four siblings from the same family (Galmiche et al. 2011), who are compound heterozygotes for a maternally derived missense mutation (identical to one detected in our patient) and a paternally inherited 255 kb deletion (Galmiche et al. 2011). The clinical features of the five known COXPD9 patients are detailed in Table 2. Persistent elevation of hepatic transaminases is reported in 4/5. All of the COXPD9 patients have demonstrated failure to thrive and a lactic acidosis, although our patient is the only one noted to have presented with a lactic acidosis in the neonatal period. Our current report expands the clinical spectrum as he is the only COXPD9 patient to have SNHL diagnosed during life, with hepatic cirrhosis and interstitial nephritis described at autopsy. The renal manifestations of

Table 2 Compares the features of the case described here with the four siblings (patients 1–4) previously described by Galmiche et al. in 2011

	Case 1	Case 2	Case 3	Case 4	Current case
HCM age at diagnosis	11 months	12 months	9 months	9 months	8 months
Hepatomegaly	+		+	+	
Elevated liver enzymes	+		+	+	+
Lactic acidosis	+	+	+	+	+
FTT	+	+	+	++	+
SNHL					+ (cochlear implant)
MRI/MRSbrain	Not reported	Not reported	Cortical hypergyria	Cortical hypergyria	Lactate peak
MRPL3 genotype	c.950>G (Pro317Arg) 255 kbp contiguous gene deletion	c.950>G (Pro317Arg) 255 kbp contiguous gene deletion	c.950>G (Pro317Arg) 255 kbp contiguous gene deletion	c.950>G (Pro317Arg) 255 kbp contiguous gene deletion	c.950>G (Pro317Arg) c.49delC p.(Arg17Aspfs*57)
MRC studies	Decreased complex I, IV, V	Decreased complex I, IV, V	Decreased complex I, IV	Decreased complex I, IV, V	Not performed
Outcome	Cardiac death at 17 months	Cardiac death at 15 months	Alive at 3 years, stable cardiomyopathy, failure to thrive, psychomotor retardation	Alive at 3 years, stable cardiomyopathy, severe failure to thrive, psychomotor retardation	Cardiac death at 11 months. Cirrhosis and interstitial nephritis
Additional findings at autopsy					Hepatic cirrhosis Interstitial nephritis

HCM hypertrophic cardiomyopathy, *FTT* failure to thrive, *SNHL* sensorineural hearing loss, *MRI* magnetic resonance imaging, *MRS* magnetic resonance spectroscopy, *MRC* mitochondrial respiratory chain, + present

mitochondrial diseases most commonly involve glomerular (e.g. focal segmental glomerulosclerosis, nephrotic syndrome) or tubular pathology (e.g. Fanconi syndrome, renal tubular acidosis) (Emma et al. 2012; O'Toole 2014). However interstitial nephritis has been reported in a range of mitochondrial disease including mtDNA defects (e.g. the common MELAS mutation m.A3243G in the *MTTL1* gene (MIM 590050)), respiratory chain assembly factors (e.g. the complex-III assembly factor *BCSL1* (MIM 603647)), defects of post-translational modification of mitochondrial aminopeptidases (e.g. *XPNPEP3* MIM613513) and most commonly disease processes associated with elevated excretion of methylmalonic acid (Emma et al. 2012; O'Toole 2014). While the interstitial nephritis in our patient was identified at autopsy, he was not exposed to any known medications or infectious agents associated with interstitial nephritis. We postulate that his evolving multisystem disease process would have included a significant renal burden if his cardiac disease hadn't intervened.

COXPD-9 deficiency due to pathogenic mutations in the *MRPL3* gene can be predicted to produce a severe multisystem disorder as observed in our patient. The advent of accessible next-generation sequencing meant that we didn't move to mitochondrial respiratory chain biopsies in our patient. The original report by Galmiche et al. demonstrated a combined decrease in activity of mitochondrial respiratory complexes I, III, IV and V, with a mild decrease in complex II on fibroblasts and muscle samples. This highlights the critical role of MRPL3 in the early steps of mitochondrial translation. Galmiche et al. also demonstrated alteration in the stability of MRPL3 and defective assembly of the large ribosomal subunit resulting from the *MRPL3* NM_007208 c.950C>G p.(Pro317Arg) variant.

The mitoribosome is a protein complex which is active within the mitochondria whose function is to facilitate the translation of mtDNA-encoded proteins. Thus the mitoribosome synthesises 13 components of the mitochondrial oxidative phosphorylation complex, which contribute to all of the mitochondrial respiratory chain complexes with the exception of complex II. At the time of writing, there are 18 patients reported with clinical phenotypes secondary to mitochondrial ribosomal subunit mutations in the *MRPL3*, *MRPL12*, *MRPL44*, *MRPS16*, *MRPS22* and *MRPS7* genes, which are summarised in Table 1. They share common

clinical features to our patient including cardiomyopathy, lactic acidosis and renal involvement. Cardiomyopathy is a common component to the phenotype mitochondrial ribosomal protein defects, having been present in all of the reported *MRPL3* and *MRPL44* cases and almost all of the reported *MRPS22* cases (Galmiche et al. 2011; Serre et al. 2013; Carroll et al. 2013; Distelmaier et al. 2015; Saada et al. 2007; Smits et al. 2011; Baertling et al. 2015). The mitochondrial ribosomal subunit disorders appear to have a severe phenotype, with death in the neonatal period in five cases and before 3 years in a further five patients (including our case). Of the remaining eight patients, one succumbed to hepatic failure aged 14, one was very severely disabled aged 5.5 years, and two had significant impairment at age 3 (Serre et al. 2013; Carroll et al. 2013; Distelmaier et al. 2015; Miller et al. 2004; Saada et al. 2007; Menezes et al. 2015; Smits et al. 2011; Baertling et al. 2015).

Perturbations in the mitoribosome translational machinery produce significant disruption to mitochondrial function, which is manifested in the severe and often lethal clinical phenotypes as demonstrated by the current cohort of COXPD9 deficiency patients.

Compliance with Ethics Guidelines

Conflict of Interest

David Coman, Carolyn Bursle, Anna Narendra, Raymond Chuk, Bruce Lewis, Rob Justo, and John Cardinal declare that they have no conflicts of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (Carroll et al. 2013). Informed consent was obtained from all patients for being included in the study.

Author Contributions

Dr. Carolyn Bursle has driven the manuscript development and been involved in patient care.

Dr. Anna Narendra has driven the manuscript development and been involved in patient care.

Dr. Raymond Chuk has driven the manuscript development and been involved in patient care.

Dr. Bruce Lewis is a senior paediatrician coordinating the patients care and contributed to the manuscript development.

Dr. Rob Justo is a paediatric cardiologist involved in the patient's care and contributed to the manuscript development.

Dr. John Cardinal is a medical scientist who has been involved in the manuscript development.

Professor David Coman is a metabolic physician coordinating care of patient and has coordinated the manuscript development and design.

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