Mitochondrial Respiratory Chain Disorder in Two Filipino Children

Mary Anne D. Chiong[1,2] and Carmencita David-Padilla[1,2]

[1]*Department of Pediatrics, College of Medicine and Philippine General Hospital, University of the Philippines Manila*
[2]*Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila*

ABSTRACT

Mitochondrial respiratory chain disorders have very diverse manifestations and can present with any symptom, in any organ at any time. Here we describe two Filipino children confirmed to have a mitochondrial respiratory chain disorder after presenting with non-specific neurologic symptoms. The first patient had Otahara syndrome and was later on found to have complex I deficiency. The second patient had the m.8993T>G mtDNA mutation that was consistent with a Leigh phenotype.

Key Words: mitochondrial respiratory chain disorder, complex I deficiency, Otahara syndrome, m.8993T>G mutation, Leigh syndrome NARP phenotype

Introduction

The mitochondrion, being the powerhouse of the cell is the organelle which supports aerobic respiration and provides energy for the metabolic pathways. Mitochondria are relics of independent bacterial intruders that took permanent residence in our cells over a billion years ago. That is why mitochondria still possess their own circular DNA (mtDNA) which encodes 13 proteins of the approximately 90 proteins of the respiratory chain. All the other proteins of the respiratory chain needed for replication, repair, transcription, translation and proper assembly and function are encoded by nuclear DNA (nDNA).[1,2] The mitochondrial respiratory chain catalyzes the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP via five complexes (Figure 1).[2] The transfer of protons creates a charge differential that leads to the production of ATP.[3] Mitochondria also play a role in cell signaling, and host several pathways such as Krebs cycle, beta oxidation and lipid synthesis. Given these fundamental functions, defects of mitochondrial function can have disastrous consequences and may result in one or more of the following: an increase of reducing equivalents in both mitochondria and cytosol, a decrease in mitochondrial ATP formation, an increase in superoxide formation, and functional impairment of metabolic pathways requiring mitochondrial respiratory chain function such as Krebs cycle and beta oxidation.[2,4]

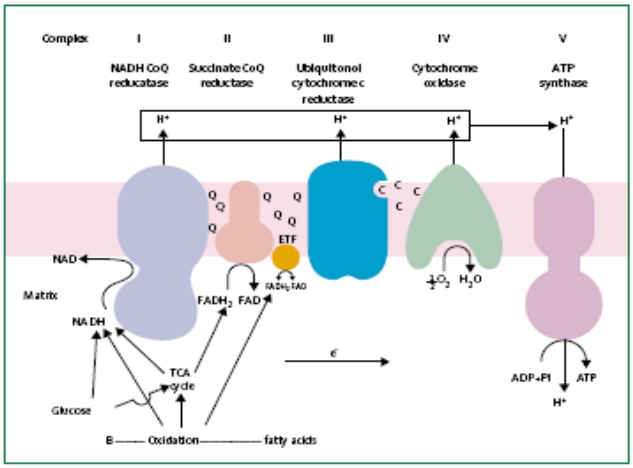

Figure 1. Complex I (NADH-ubiquinone reductase) carries reducing equivalents from NADH to the ubiquinone pool, complex II (succinate-ubiquinone reductase) carries reducing equivalents from FADH2 to CoQ pool, complex III (ubiquinol-cytochrome c reductase) carries electrons from the CoQ pool to cytochrome c, complex IV (cytochrome c oxidase, COX) catalyzes the transfer of reducing equivalents from cytochrome c to molecular oxygen producing water and complex V (ATP synthase) allows proton to flow back into the mitochondrial matrix and uses the released energy to synthesize ATP.[2]

The first mitochondrial disorder was recognized in 1961. Pediatric cases were recognized rather later, but since the 1980s, presentations of pediatric mitochondrial disorders have been widely reported. The great variety of symptomatology and, in many of the cases, rapidly progressive course, are well known because of the major involvement of high rate aerobic metabolism in most organs, specifically the brain, heart and skeletal muscles.[2,5] Mitochondrial disorders are thus a genetically, biochemically, and clinically heterogenous group of

Corresponding author: Mary Anne D. Chiong, MD
Institute of Human Genetics
National Institutes of Health
University of the Philippines Manila
625 Pedro Gil Street, Ermita, Manila 1000 Philippines
Telephone: +632 5261725
Email: maryannechiong@gmail.com

disorders involving oxidative phosphorylation. The estimated incidence is 1:7000-10,000 live births.[6,7,8,9] Here we report the first two confirmed cases of mitochondrial respiratory chain disorder in two Filipino infants.

Clinical Reports

Patient 1

This patient was a 3-month-old female born to non-consanguineous parents of Chinese descent. She was delivered full term after a pregnancy complicated by urinary tract infection and bronchial asthma during the first trimester and premature contractions at 33 weeks age of gestation. The pregnancy was carried on to term, with a normal spontaneous delivery and a birth weight of 2.4 kg, length of 47 cm and with good Apgar scores. The neonatal course was complicated by jitteriness during the first 24 hours of life that persisted even after correction of hypocalcemia and treatment for sepsis. Cranial ultrasound showed non-specific right thalamus mineralizing angiopathy with bilateral grade 1 germinal matrix hemorrhage and a right subependymal cyst. EEG was normal. Expanded newborn screening (NSW Newborn Screening Program, the Children's Hospital at Westmead) was also normal. A heart murmur was likewise noted which on 2D-echocardiography revealed a patent ductus arteriousus, mild to moderate tricuspid regurgitation and right ventricular and right atrial hypertension. She was discharged asymptomatic on the second week of life.

At 25 days of life, she was noted to have seizure occurring mostly in sleep with initial eye version to the left and subsequent head and eye version to the right. Repeat EEG at 2 months of age showed a burst-suppression pattern with considerations of either early infantile epileptic encephalopathy (EIEE/ Otahara syndrome) or early myoclonic encephalopathy (EME). Clonazepam and Valproic acid were started. MRI at this time showed an increased signal intensity on white matter symmetrically. She was readmitted at 3 months of age due to persistence of seizures. A video EEG showed frequent electrographic seizures with onset independently from the right or left posterior, temporal and parietal regions with evolution to the other hemisphere in alternating fashion, each lasted 60-120 seconds with a minimum of 12 seizures in a 30 minute period. She was then transferred to PICU where the seizures remained intractable despite intravenous midazolam. Ophthalmologic evaluation showed optic nerve hypoplasia on the left. Her clinical course was complicated by liver problems, splenomegaly, hypothyroidism and drug allergy. Investigations included a urine amino acid screen which showed increase in alanine, urine organic acid screen which showed grossly increased lactate, slightly increased Kreb's cycle metabolites and presence of compounds that were suggestive of ketosis. Plasma lactate was 2.8 umol/L. A mitochondrial respiratory chain disorder was suspected and the patient was started on a vitamin cocktail consisting of L-carnitine (100 mg/kg/day), Coenzyme Q (5 mg/kg/day), Vitamin K (1 mg/kg/day), Vitamin C (60 mg/kg), Thiamine (37 mg/kg/day), Riboflavin (18 mg/kg/day), Biotin (5 mg/day), Folic acid (5 mg/day), alpha lipoic acid (25 mg/day) and Pyridoxine (150 mg/day). She initially improved and seizures were noted to decrease in frequency and duration with topiramate, levetiracetam and clonazepam. However, she subsequently developed nosocomial gram negative sepsis and candidiasis, upper gastrointestinal bleeding, pulmonary hemorrhage and hemothorax. CT scan of the abdomen showed hepatomegaly with diffuse fatty replacement and splenomegaly. She died at 5 months of age. Respiratory chain enzymes (Mitochondrial Laboratory, Victorian Clinical Genetics Services and Murdoch Childrens Research Institute) in skeletal muscle homogenate were low for complex I relative to protein (21% of control mean) and borderline low for other enzymes (38-60%). Complex I was borderline low relative to citrate synthase and complex II (36% and 35% of control means). In the liver homogenate, respiratory chain enzymes were borderline low for complex I (39%). Complex I was low relative to citrate synthase and relative to complex II (15% and 21%, respectively). In conjunction with the clinical features, metabolic investigations and with the skeletal muscle and liver enzymology, the above results were diagnostic of a respiratory chain complex I deficiency.

Patient 2

This was a 4-month-old female patient born to non-consanguineous parents after a pregnancy complicated by UTI during the first trimester. She was delivered full term by repeat cesarean section with a birth weight of 2.95 kg and good Apgar scores. Her perinatal and neonatal course were unremarkable. Developmentally, she was noted to have slower motor development compared to her older sister. At 4 months of age, she turned to her side occasionally, brought her hands to her mouth, had a social smile and could focus and track but had prominent head lag and could not lift her head while on prone. At the same age, she was rushed to a local emergency room because of difficulty of breathing. Chest x-ray and otorhinolaryngology consult showed normal findings. Blood gas showed metabolic acidosis with an increased anion gap of 22. Plasma lactate levels were 9.6 and 7.7 umol/L. She also had elevated LDH, CK-MB and LFT's. Urine organic acid analysis indicated gross lactic acidosis, ketosis and Kreb's cycle metabolites. A mitochondrial respiratory chain disorder was then suspected. On physical examination, she was lethargic with weight, height and head circumference that were appropriate for age. Liver was palpable 4 cm below the right costal margin. The rest of the physical examination was normal. Neurologic examination showed head lag and truncal hypotonia, normal deep tendon reflexes, bilateral

Babinski and 1-2 beats clonus. Cranial MRI showed signal abnormality within the bilateral putamen and caudate nucleus with associated lactate peak on spectroscopy. Vision and hearing evaluation were normal as well as 2D echocardiography. Renal evaluation was consistent with renal tubular acidosis. Mutation analysis of DNA sample from skin fibroblasts (Baylor College of Medicine, Medical Genetics Laboratory) showed a m.8993T>G mutation in the ATPase6 gene of the mitochondria. She was found to have 93% heteroplasmy for the m.8993T>G mutation in her blood and 33% heteroplasmy in her skin. She had a couple of metabolic decompensations characterized by acidosis and hyperlactacidemia during a bout of viral infection that were easily corrected by bicarbonate correction, fluids and supportive management. At the time of this report at 10 months of age, her development is progressing steadily albeit slowly as she undergoes physical therapy and is maintained on thiamine, coenzyme Q, carnitine and vitamin E.

Discussion

A defect of oxidative phosphorylation can be suspected in patients with an unexplained combination of neuromuscular and/or non-neuromuscular symptoms, progressive course, and involvement of seemingly unrelated organs. These clinical symptoms, either isolated or in combination, may occur at any stage.[4] These characteristics were seen in our two patients. Patient 1 presented in the neonatal period with severe encephalopathy associated with intractable seizures. Newborns with mitochondrial respiratory chain disorders who presented initially with symptoms pertaining to the nervous system were found to evolve into a multisystem disorder on long-term follow-up and a remarkably high mortality rate was reported in the first 3 months of life.[5] Although many of our patient's associated features can be attributed to her severe disease state and sepsis, her liver problems, hypothyroidism and ocular manifestations are all likely to have been part of the mitochondrial respiratory chain disorder.[3] The second patient presented with hyperlactacidemia on the background of developmental delay and muscular hypotonia. Likewise, the latter neurologic and muscular symptoms are among the commonest manifestations of a mitochondrial disorder.[4]

Plasma lactate, although not specific, is a helpful marker for a possible mitochondrial respiratory chain disorder. However, it is important that correct sample collection and handling are done to avoid secondary causes of elevated lactate. Persistently elevated lactate is very suggestive of respiratory chain deficiency. This is brought about by the functional impairment of tricarboxylic acid (TCA) cycle due to the excess of NADH and the lack of NAD^+ leading to an increase in ketone body (β-hydroxybutyrate/aceto-acetate) and lactate/pyruvate molar ratios with secondary elevation of blood lactate.[3] Elevated lactate was seen in both patients. Likewise, their urine organic acid analysis showed gross lactic acidosis and ketosis with elevated levels of TCA metabolites. These findings heightened the likelihood of a mitochondrial respiratory chain disorder in the two patients in concurrence with their clinical findings. It must be noted however, that absence of lactic acidosis does not rule out a respiratory chain defect, especially in somewhat later presentations.

Enzyme analysis in intact mitochondria and molecular analysis of nuclear or mitochondrial DNA mutations are two of the major tools that will help in the establishment of diagnosis for a mitochondrial respiratory chain disorder.[8] Patient 1 showed low activity of respiratory chain enzymes in complex I of both the skeletal and liver homogenates. Patient 2 on the other hand had a confirmed mitochondrial DNA mutation in skin fibroblasts which harbored the mtDNA m.8993T>G mutation.

Complex I deficiency is the most commonly observed mitochondrial respiratory chain defect.[5,10] A diversity of clinical phenotypes has been reported to occur in complex I deficient patients, which can be classified into several main clinical phenotypes: severe lactic acidosis, Leigh syndrome, neonatal cardiomyopathy with lactic acidosis, leukodystrophy with macrocephaly, hepatopathy with renal tubulopathy and a group of miscellaneous unspecified mitochondrial encephalomyopathies.[11,12,13] Patient 1 presented with encephalopathy characterized by severe neonatal seizures suspected to be a possible Otahara syndrome. Her lactic acidosis was only determined once and it was only mildly elevated. Her MRI findings as well as clinical course were not compatible with Leigh syndrome because Leigh syndrome usually manifests with early onset, progressive neurologic disorder characterized by motor and intellectual developmental delay, signs of brainstem and basal ganglia involvement and increased lactate levels in blood and/or CSF.[14] These features were not seen in patient 1. However, a rare manifestation of complex I deficiency has recently been reported in a neonate who presented with Otahara syndrome that was very similar to that seen in our patient.[14] Since mitochondrial diseases are known to be clinically very heterogenous, Otahara syndrome although a rare complex I phenotype should be considered as one of the rare forms of presentation of a mitochondrial disease.[15] Patient 2 had the m.8993T>G mutation associated with Leigh syndrome and neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) phenotypes. The clinical presentation in individuals carrying this mitochondrial DNA mutation varies with the degree of m.8993T>G heteroplasmy in affected tissues.[16] Heteroplasmy is a unique characteristic of mitochondrial genetics wherein coexistence of wild type and mutant mtDNA varies in proportion in different tissues.[2] For this specific mutation, a phenotypic gradient exists in which mutation heteroplasmy <60% typically does not result

in clinical symptoms; mutation heteroplasmy between 60-75% is frequently associated with Retinitis Pigmentosa (RP), 75-90% with Neuropathy, Ataxia, and Retinitis Pigmentosa (NARP) and high level mutation heteroplasmy usually greater than 90% is commonly associated with Leigh syndrome.[16] Based on the results of patient 2, it is encouraging that her level of heteroplasmy in the skin is low but it is impossible to know what her levels are in the brain and other internal organs. Thus, it is difficult to classify at this point whether she belongs to the NARP or Leigh spectrum of phenotypes. It is likewise difficult to predict how this will affect her in the future. This complexity has been shown in a family described to have variable disease severity and tissue mitochondrial DNA T8993G mutant loads.[16,17] The siblings who had high mutant loads in all tissues (>90%) had features of NARP and LS while the one who had high mutant load in tissues (93%) derived from endoderm and mesoderm but had a lower proportion of mutant mtDNA in tissue derived from ectoderm (hair bulb) presented only with speech delay and was attending normal school. Given the shared embryonic origin of hair bulbs and the brain, it was speculated that mutant load in hair bulbs could be a reflection of the brain mutant load. This type of investigation has not yet been done on patient 2, thus it is very difficult to predict at present how severe her neurologic involvement is, although there is more evidence that she falls within the spectrum of Leigh disease because of the diffuse basal ganglia changes and developmental delay in nearly infancy.

Since the mitochondrial respiratory chain is controlled by both nuclear and mitochondrial genes, along with the diversity of clinical manifestations, genetic counseling is a considerable challenge. Mitochondrial diseases caused by nuclear gene mutations will be transmitted by Mendelian inheritance. On the other hand, the inheritance of primary mtDNA mutations will be maternal, i.e. from the mother to all offspring, and subsequently transmitted by the daughters alone. Finally, in some cases, particularly those individuals who have large deletions of the mitochondrial genome, as seen in Kearns-Sayre syndrome, the deletion usually arises as a *de novo* event and has a very low recurrence risk.[18]

No mutation analysis was done on Patient 1, thus the genetic basis and recurrence risk for the family are uncertain, however empirical recurrence risks have been calculated in complex 1 deficiency at 23-27%.[18] Likewise, respiratory chain complex 1 deficiency has been found to be mostly inherited in an autosomal recessive manner, thus, a 25% recurrence risk each pregnancy may be given as an estimate.[19,20]

The m.8993T>G mutation is one of the most common mtDNA mutations diagnosed in children which shows a strong correlation between mutant load and symptoms. These features allowed the generation of logistic regression models relating to maternal blood mutant load and risk of having a severely affected child and probability of a severe outcome in an individual based on the measured mutant load.[21] For the family of patient 2, the mother has yet to be tested for the level of heteroplasmy in her blood for recurrence risk predictions. Some guidance however, can be obtained from the recurrence risks predicted by White et al, i.e., the corresponding proportion of healthy oocytes at maternal blood mutant loads of 60%, 70% and 80% would be 32%, 23% and 16%, respectively.

There is currently no satisfactory therapy for respiratory chain disorders. Treatment remains largely symptomatic and does not significantly alter the course of the disease. It includes avoidance of drugs and procedures known to have detrimental effects, slow infusion of sodium bicarbonate during attacks of lactic acidosis and a dietary recommendation that include a high lipid, low carbohydrate diet specifically in patients with complex I deficiency.[3] Various vitamins and co-factors such as thiamine, carnitine, riboflavin, pantothenic acid have been used to help increase the activity of the different complexes. Since defects of the respiratory chain also result in the increased production of free radicals, the use of antioxidants such as vitamin E, lipoic acid and coenzyme Q also has a sound basis. However, these various therapies have proved helpful only in a very few isolated cases and possibly effective in the short term.[22] Over the last decade, there has been little progress in the development of novel treatments for nuclear-encoded disorders of the respiratory chain, but a number of strategies are currently being explored for the treatment of primary mtDNA abnormalities such as delivery of a normal version of the affected gene into the mitochondria, altering the level of heteroplasmy, alternative methods of manipulating the level of mtDNA and preferential expansion of wild type mtDNA through recruitment of satellite cells that contain little or no mtDNA.[23]

In summary, we report two Filipino children who primarily presented with neurologic and muscular symptoms along with lactic acidosis. They were later on confirmed to have a mitochondrial respiratory chain disorder based on enzymology and or mutation analysis The first patient was a severe case of complex I deficiency leading to early death in the neonatal period. Patient 2 had the common m.8993T>G mtDNA mutation that is consistent with either a Leigh or NARP phenotype, although clinically the former is more likely. Her disease has progressed and she developed severe seizures in recent times. She had regression in her previously learned skills and has become more hypotonic. Her current management remains supportive. Further investigations have to be done on the family members of both patients so that proper genetic counseling can be addressed.

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