A patient with congenital hyperlactataemia and Leigh syndrome: an uncommon mitochondrial variant

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We report an uncommon mitochondrial variant in a baby girl with congenital hyperlactataemia and Leigh syndrome. The patient presented with a single episode of generalised clonic convulsion at day 19, and was found to have isolated and persistent hyperlactataemia ranging from 3.34 to 9.26 mmol/L. She had elevated serum lactate-to-pyruvate ratios of up to 35 and high plasma alanine concentration, indicative of a respiratory chain defect. At the age of 8 months, she developed evolving neurological and imaging features compatible with Leigh syndrome. Genetic testing for common mitochondrial DNA mutations, large mitochondrial DNA deletions, and selected nuclear genes was negative. Further analysis of lymphocyte mitochondrial DNA by sequencing revealed an uncommon heteroplasmic variant, NC_012920.1(*MT-ND5*):m.13094T>C (p.Val253Ala), which was previously shown to reduce complex I activity. In patients in whom there was a high suspicion of mitochondrial disorder, entire mitochondrial DNA analysis may be warranted if initial screening of common mitochondrial DNA mutations is negative.

Introduction

Mitochondrial disorders have protean clinical manifestations. Although organ systems with high energy demand like the nervous system and muscle are more commonly affected, diagnosis of mitochondrial disorders cannot be based solely on clinical features, as they may be non-specific. Diagnosis is often made even more challenging by the genetic heterogeneity of mitochondrial disorders, which can be caused by mutations in both mitochondrial genome and at least 150 nuclear genes involved in mitochondrial biogenesis, structure, and function.[1] When a mitochondrial disorder is suspected based on clinical, biochemical, and imaging evaluations, screening for the recurrent mitochondrial DNA mutations and large deletions is usually indicated. This is particularly true of well-established conditions such as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy associated with ragged-red fibres), and Leigh syndrome. However, these common mutations account for only a small proportion of mitochondrial disorders. One study reported that the detection rate of these recurrent mutations and large deletions in patients with suspected mitochondrial disorders was only 6%.[2] If screening of common mitochondrial DNA mutations is negative, sequencing the mitochondrial genome and selective candidate nuclear genes may be warranted. Here, we report an uncommon mitochondrial DNA variant in a baby girl who initially presented with congenital hyperlactataemia at day 19 and developed evolving neurological features of Leigh syndrome at 8 months old.

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Case report

The patient, the second child of non-consanguineous Chinese parents, was born full term by normal spontaneous delivery. Aged 19 days, she presented with a single generalised clonic convulsion lasting minutes, but was otherwise well, and no similar episode ensued during her hospitalisation. Physical examination was unremarkable. Laboratory investigations revealed isolated hyperlactataemia ranging from 3.34 to 9.26 (reference range, 0.50-2.20) mmol/L. The patient had neither hypoglycaemia nor hyperammonaemia. At 1 month, she had a second generalised clonic convulsion which resolved without treatment. She had persistent hyperlactataemia. The lactate-to-pyruvate ratio was 35 at a plasma lactate of 7.48 mmol/L. Urine organic acid profiling, plasma amino acid profiling, and plasma acylcarnitine profiling performed at 1 month were unremarkable, except for an increased plasma alanine concentration of 706 (reference range, 143-439) μmol/L. Electroencephalography performed at the age of 6 weeks showed symmetrical and continuous background activity with no burst suppression pattern. Sporadic sharp

一名有先天性高乳酸鹽血症和Leigh綜合徵的病人：一種罕見的線粒體變異

本文報告一宗患有罕見線粒體變異的病例。患者為一名女嬰，她同時有先天性高乳酸鹽血症和Leigh綜合徵。病人出生後第19日出現單次發作的全身陣攣性抽搐。她乳酸鹽血水平偏高，維持在3.34至9.26 mmol/L之間，而血清的乳酸/丙酮酸的比率最高達至35，並有高血漿丙氨酸濃度，顯示病人有呼吸鏈功能缺陷。病人八個月大時，漸漸出現與Leigh綜合徵吻合的神經系統徵狀和影像學特徵。常見的線粒體DNA突變、大型線粒體DNA缺失，以及特選核基因測試結果均呈陰性。進一步的淋巴細胞線粒體DNA基因測試發現一種罕見的異質性變異，NC_012920.1(MT-ND5):m.13094T>C (p.Val253Ala)，過往研究顯示這變異會減少複合物I活性。如果臨床徵狀和檢查顯示病人很可能患有線粒體疾病，而常見的線粒體DNA突變的測試結果呈陰性的話，便可能值得考慮替病人進行整個線粒體DNA基因分析。

hypotonia, and gastroesophageal reflux. At the age of 7 months, developmental delay was noted, there being no reaching out and sitting with support. Other evolving neurological features included bilateral ptosis, nystagmus, ophthalmoplegia, and hypotonia. The patient's head circumference, height, and body weight were at the 10th percentile. She also had subtle dysmorphic features, with mild micrognathia and hypertelorism. A repeat brain MRI at 8 months old showed bilateral symmetrical T2-hyperintense signals in the periaqueductal grey matter, posterior limbs of the internal capsule, the cerebral peduncles, middle cerebellar peduncles, and ventral medulla oblongata (Fig b). Proton magnetic resonance spectroscopy of the brain demonstrated a prominent lactate peak corresponding to areas with abnormal T2-weighted signals. The parents refused any invasive procedure and thus muscle biopsy was not performed for histological examination or assay of mitochondrial respiratory chain complex activity.

waves, which were normal for her age, were seen, and no ictal discharge was noted. The first magnetic resonance imaging (MRI) of the brain performed at the age of 2 months yielded no abnormality (Fig a). At the age of 4 months, the patient started to have poor feeding, failure to thrive, generalised

Molecular genetic studies were performed after obtaining informed consent from the parents. Genomic DNA was extracted from peripheral blood. Screening of common mitochondrial DNA mutations by polymerase chain reaction (PCR)/ restriction fragment length polymorphism (RFLP)
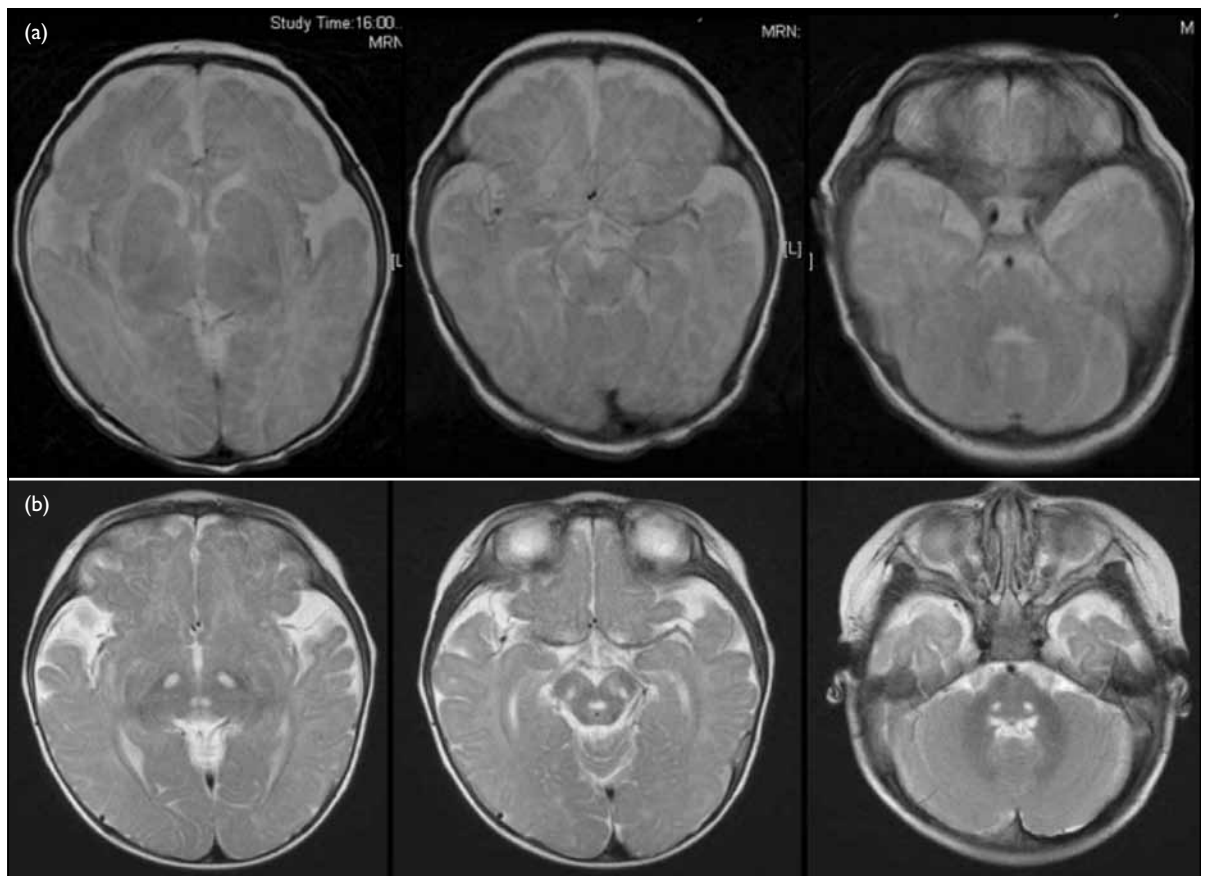

FIG. Magnetic resonance imaging of the brain: (a) performed at 2 months old shows no abnormality, and (b) performed at 8 months old shows bilateral symmetrical T2-hyperintense signals in periaqueductal grey matter, posterior limbs of the internal capsule, cerebral peduncles, middle cerebellar peduncles, and ventral medulla oblongata

and testing of large mitochondrial DNA deletions by long-range PCR were negative. Genetic analyses of nuclear genes including polymerase, DNA, gamma (*POLG*), and pyruvate carboxylase (*PC*) by PCR and direct DNA sequencing of the coding exons and the flanking introns were also negative. As there was a high suspicion of mitochondrial disorder, PCR and sequencing of part of the mitochondrial DNA, involving regions flanking the common point mutations, using the same primers in RFLP analysis was performed. In this patient, an uncommon heteroplasmic variant, NC_012920.1(*MT-ND5*):m.13094T>C (p.Val253Ala), was identified. The pathogenic effect of this variant on complex I activity had been previously demonstrated.[3] The variant was verified by RFLP analysis (*AluI*) of PCR products and by recourse to amplification refractory mutation system, for which primer sequences and protocols are available upon request. Accurate quantification of the heteroplasmy level was not performed, but the mutant load in lymphocytes was observed to be more than 50%, based on the direct sequencing result. The variant was absent in maternal lymphocytes by direct sequencing, RFLP, and by amplification of the refractory mutation system.

The patient was admitted to hospital again at the age of 11 months for apnoea. Subsequently, her clinical condition deteriorated progressively with respiratory failure, autonomic dysfunction with tachycardia and hypertension, spasticity and later decerebrate rigidity. She finally succumbed from pneumonia at the age of 25 months.

Discussion

Primary congenital lactic acidosis can be caused by a number of inborn errors of metabolism, including defects in pyruvate metabolism, gluconeogenesis disorders, respiratory chain defects of either nuclear or mitochondrial DNA origin, and Krebs cycle defects. Several other inborn errors of metabolism, such as organic acidurias and fatty acid oxidation defects, can also lead to secondary hyperlactataemia as a result of interference in coenzyme A metabolism at the level of pyruvate dehydrogenase and PC. Common acquired causes of lactic acidosis like tissue hypoxia should always be ruled out before considering inherited diseases. Biochemical investigations together with clinical features could help narrow down the various differential diagnoses of congenital lactic acidosis. One of the useful indicators to guide further investigation is the plasma lactate-to-pyruvate molar ratio, which reflects the cytoplasmic NADH-to-NAD+ ratio and hence acts as a surrogate measure of the redox state in cytosols. A normal lactate-to-pyruvate ratio (conventionally below 25) points to pyruvate dehydrogenase complex (PDHC) deficiency, PC deficiency type A, or gluconeogenesis defects; an elevated ratio suggests a defective respiratory chain, a Krebs cycle defect, or PC deficiency type B. The latter condition is also characterised by a normal beta-hydroxybutyrate-to-acetoacetate ratio on top of a raised lactate-to-pyruvate ratio. At the established optimal cutoff of 23.3, however, Debray et al[4] found that the blood lactate-to-pyruvate ratio had relatively low sensitivity and specificity of 77% and 91%, respectively for differentiating respiratory chain defects from pyruvate dehydrogenase deficiency. The same group also demonstrated an improved diagnostic accuracy of lactate-to-pyruvate ratio at higher lactate concentrations. When the blood lactate level was above 5 mmol/L, the sensitivity and specificity of the lactate-to-pyruvate ratio at an optimal cutoff of 25.8 improved to 96% and 100%, respectively.[4] The elevated lactate-to-pyruvate ratio of 35 in our patient with a plasma lactate of 7.48 mmol/L was highly suggestive of a respiratory chain defect. In addition, a plasma alanine concentration of higher than 450 µmol/L was also indicative of mitochondrial disorder, reflecting chronic pyruvate accumulation.[5]

Our patient first presented with isolated and persistent congenital hyperlactataemia. Screening for common mitochondrial DNA point mutations and large deletions at that time were all negative. It was not until later months that the patient developed evolving neurological and imaging findings compatible with Leigh syndrome. Leigh syndrome, also known as subacute necrotising encephalomyelopathy, is characterised by focal, bilateral, symmetrical deep grey nuclei lesions particularly involving basal ganglia and periaqueductal grey matter.[6] Most patients have varying degrees of encephalopathy, basal ganglia, and brainstem dysfunction. Similar to all mitochondrial disorders, Leigh syndrome is genetically heterogeneous but some subtypes are known to be more common. If the initial screening for common mitochondrial DNA mutations is negative, genetic testing for PDHC deficiency, cytochrome c oxidase deficiency (eg *SURF1* mutations), complex I deficiency, and mitochondrial DNA depletion syndrome (eg *POLG* mutations), and sequencing of the entire mitochondrial DNA has been suggested.[1] Genetic testing of *POLG* and *PC* was performed in our patient, with negative results. Notably, *POLG* is the most frequently implicated nuclear gene causing mitochondrial diseases.[1] Although Alpers' syndrome is the most common form of *POLG* deficiency, the clinical manifestations of *POLG* mutations can be extremely heterogeneous and include the Leigh syndrome.[7,8] Pyruvate carboxylase is important in generation of Krebs cycle intermediates, gluconeogenesis and lipogenesis. Furthermore, PC deficiency should also be considered in infants presenting with lactic acidosis and neurological abnormalities. The lactate-to-pyruvate ratio is

high in type B PC deficiency, and can be normal to moderately increased in type A deficiency. Although PC deficiency is a gluconeogenesis disorder, hypoglycaemia may not always be a prominent feature, unless during fasting.[9]

The heteroplasmic variant, NC_012920.1(*MT-ND5*):m.13094T>C (p.Val253Ala) identified in our patient was newly described in 2009 in a 7-year-old child presenting with ataxia, chronic progressive external ophthalmoplegia, and MRI findings suggestive of mitochondrial encephalopathy.[3] The mutant load in Valente et al's patient[3] was 50% heteroplasmy in skeletal muscle and 40% heteroplasmy in lymphocytes. By contrast, our patient had a different phenotype and presented with congenital hyperlactataemia and later evolved into the Leigh syndrome. Accurate quantification of the heteroplasmy level was not performed in our patient as neither muscle nor skin fibroblast samples were available for study. The mutant load in lymphocytes was estimated to be more than 50% based on the direct sequencing result. The variant in *ND5* gene, causing a change of valine to alanine at position 253, affects a highly conserved residue. Besides, the pathogenic effect of the variant on complex I activity had also been demonstrated, showing that the complex I–to–citrate synthase activity ratio was highly correlated with the percentage of the variant. Moreover, a 50% heteroplasmy would be expected to reduce complex I activity by 49% compared with the wild type.[3] Nevertheless, Valente et al[3] also pointed out that the skeletal muscle morphology and respiratory chain activity, including that of complex I, were normal in their patient.

The unique characteristics of mitochondrial DNA, namely maternal inheritance, heteroplasmy, tissue variation and threshold effect, pose significant challenges to the provision of genetic counselling and reproductive planning to affect individuals and their families. Even though the mother of a proband usually harbours the mitochondrial DNA mutation and she may or may not have symptoms depending on mutant load and tissue distribution, there could be de-novo mitochondrial mutation in a proband. In this case, the variant NC_012920.1(*MT-ND5*):m.13094T>C (p.Val253Ala) was not detected in the mother's lymphocyte DNA. Nevertheless, mitochondrial DNA mutation may display various degrees of heteroplasmy in different tissues, including oocytes. The result could not rule out the possibility of a detectable mutant load in her other tissues, such that future pregnancies could still be at risk of inheriting the variant and developing symptoms.

Mitochondrial oxidative phosphorylation is under dual control of both mitochondrial and nuclear genomes that encode respiratory chain subunits, assembly factors, and also other proteins involved in mitochondrial DNA biosynthesis, structure, and function. Although there are some recognisable syndromes, the yield of symptom-based protocols linking directly to genetic testing of selective genes or mitochondrial point mutations is still limited as a result of high clinical and genetic heterogeneity. Thorburn[10] reported that mitochondrial DNA mutations accounted for 20 to 25% of mitochondrial disorders. Other studies involving entire mitochondrial DNA sequencing or chip-based screening of complete mitochondrial DNA identified mutations in 14 to 25% of suspected mitochondrial disorder patients who were negative for the common mitochondrial DNA point mutations or deletions.[3,11] As a result, complete mitochondrial DNA analysis could be warranted for patients with suspected respiratory chain defects, particularly when screening of the recurrent mitochondrial DNA mutations and genetic testing of *POLG* (the most frequently mutated nuclear gene causing mitochondrial disorders) are negative.

In conclusion, we identified an uncommon mitochondrial DNA variant, NC_012920.1(*MT-ND5*):m.13094T>C (p.Val253Ala) in a baby girl with congenital hyperlactataemia, who developed Leigh syndrome after several months. An elevated lactate-to-pyruvate ratio, especially at high lactate levels (>5 mmol/L) and an isolated increase in plasma alanine level (>450 µmol/L) very likely point to a respiratory chain defect. Assays of respiratory chain complex activities, though not available in this patient, constitute an important means of evaluating patients with suspected mitochondrial disorders. Entire mitochondrial DNA analysis appears warranted if there is a high suspicion of such a disorder when initial tests for common mitochondrial DNA mutations are negative.

References

1. Wong LJ. Molecular genetics of mitochondrial disorders. Dev Disabil Res Rev 2010;16:154-62. **cross** ref
2. Liang MH, Wong LJ. Yield of mtDNA mutation analysis in 2000 patients. Am J Med Genet 1998;77:395-400. **cross** ref
3. Valente L, Piga D, Lamantea E, et al. Identification of novel mutations in five patients with mitochondrial encephalomyopathy. Biochim Biophys Acta 2009;1787:491-501.
4. Debray FG, Mitchell GA, Allard P, Robinson BH, Hanley JA, Lambert M. Diagnostic accuracy of blood lactate-to-pyruvate molar ratio in the differential diagnosis of congenital lactic acidosis. Clin Chem 2007;53:916-21. **cross** ref
5. Mitochondrial Medicine Society's Committee on Diagnosis, Haas RH, Parikh S, Falk MJ, et al. The in-depth evaluation

of suspected mitochondrial disease. Mol Genet Metab 2008;94:16-37. cross ref

6. Friedman SD, Shaw DW, Ishak G, Gropman AL, Saneto RP. The use of neuroimaging in the diagnosis of mitochondrial disease. Dev Disabil Res Rev 2010;16:129-35. cross ref

7. Taanman JW, Rahman S, Pagnamenta AT, et al. Analysis of mutant DNA polymerase gamma in patients with mitochondrial DNA depletion. Hum Mutat 2009;30:248-54. cross ref

8. The National Institute of Environmental Health Sciences. Human DNA polymerase gamma mutation database. Available from: http://tools.niehs.nih.gov/polg. Accessed 23 April 2012.

9. De Meirleir LJ, Van Coster R, Lissens W. Disorders of pyruvate metabolism and the tricarboxylic acid cycle. In: Fernandes J, Saudubray JM, van den Berghe G, Walter JH, editors. Inborn metabolic diseases. Diagnosis and treatment. 4th ed. Würzburg: Springer; 2006: 161-74. cross ref

10. Thorburn DR. Mitochondrial disorders: prevalence, myths and advances. J Inherit Metab Dis 2004;27:349-62. cross ref

11. van Eijsden RG, Gerards M, Eijssen LM, et al. Chip-based mtDNA mutation screening enables fast and reliable genetic diagnosis of OXPHOS patients. Genet Med 2006;8:620-7. cross ref

Answers to CME Programme
Hong Kong Medical Journal June 2013 issue

Hong Kong Med J 2013;19:203–6

I.   Prevalence of abnormal Papanicolaou smears in female sex workers in Hong Kong

A	1. True	2. False	3. True	4. True	5. False
B	1. False	2. True	3. False	4. True	5. False

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II.   Aspirin desensitisation for Chinese patients with coronary artery disease

A	1. True	2. True	3. False	4. False	5. False
B	1. True	2. True	3. False	4. True	5. True