INTRODUCTION

- Mitochondrial diseases (MDs) occur in all age groups and present with several clinical features. However, cases of neonatal-onset follow different courses from those that begin in infancy or adulthood, with many of them becoming serious.

- Studies that include a large number of patients with neonatal-onset MD are limited\(^1\)-\(^2\), and its clinical and molecular features have not been fully elucidated due to the heterogeneity of MD.

- Here, in a large retrospective observational study in Japan, we analyzed 281 patients with neonatal-onset MD diagnosed by both biochemical and genetic approaches to clarify its clinical features, molecular diagnosis, and prognosis.

- This observational study of neonatal-onset mitochondrial disease in the largest cohort ever is the first in Asia.

- There are no reports on factors affecting the prognosis of neonatal-onset MD. This is the first statistical analysis.


METHODS

Patients

<table>
<thead>
<tr>
<th></th>
<th>Neonatal-onset MD (n = 294)</th>
<th>Excluded (n = 13)</th>
<th>Enrolled (n = 281)</th>
<th>Biochemical approach (n = 271)</th>
<th>Genetic approach* (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Diagnosed in Chiba Children’s Hospital from January 2004 to March 2020</td>
<td>(Clinical course was unknown)</td>
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<td>First line</td>
<td>MRC enzyme assay (n = 182)</td>
<td>OCR measurement (n = 89)</td>
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<tr>
<td>MRC: mitochondrial respiratory chain</td>
<td>(No decrease in MRC enzyme activity)</td>
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<td>OCR: oxygen consumption rate</td>
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*The genetic approach was used when a sibling had a confirmed case based on molecular diagnosis or when there were no samples available for biochemical diagnosis.

Disease types

Multisystem MD (MsMD)\(^*\), Leigh syndrome (LS), Cardiomyopathy (CM), Hepatic disease (HD)

*MsMD: primarily associated with multiple organ failure and hyperlactatemia that could not be classified as LS, CM, or HD.
Variant analysis of the genes responsible for MD

Genomic DNA
• Sanger sequencing
• Whole mitochondrial DNA sequencing
• Targeted resequencing of whole mitochondrial DNA

Exons of nuclear-encoded genes that cause MDs
• Whole-exome sequencing
• High-density oligonucleotide arrays to identify large chromosomal deletions

Statistical analysis
○ Multiple comparison of the Fisher’s exact test using the Bonferroni method.
○ Multivariate analysis to identify independent predictors for all-cause mortality.
  • Kaplan–Meier analysis for survival curves
  • Log-rank tests to identify independent predictors for all-cause mortality.
  • Preterm birth, small-for-gestational-age (SGA), hyperlactatemia; early onset (within two days of birth), molecular diagnosis, MD with specific treatment options; each disease type
  • Cox proportional hazards regression analyses
    factors significant at the level of P < 0.10 in the log-rank test.
(P < 0.05 were considered statistically significant)

RESULTS

Characteristics of each disease type in 281 newborns with neonatal-onset MD

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MsMD (n = 194)</th>
<th>LS (n = 26)</th>
<th>CM (n = 38)</th>
<th>HD (n = 23)</th>
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<tbody>
<tr>
<td>Male</td>
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<tr>
<td>Preterm delivery†</td>
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<tr>
<td>Deceased (≤ 28 days after birth)§</td>
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<tr>
<td>Deceased (&gt; 28 days after birth)§</td>
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<td>Onset (0-1 day after birth)§</td>
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<tr>
<td>Onset (2-28 days after birth)§</td>
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<tr>
<td>Hyperlactatemia (&gt; 2.1 mM)§</td>
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<tr>
<td>Diagnosed by biochemical approach</td>
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<td>Diagnosed by MRC enzyme activity</td>
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<td>Diagnosed by OCR§</td>
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<tr>
<td>Diagnosis by genetic approach</td>
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* There is a significant difference in the multiple comparisons of Fisher’s exact tests using the Bonferroni method between four disease types
* Number of patients with available information only
* No decrease in MRC enzyme activity

MsMD, multisystem mitochondrial disease; LS, Leigh syndrome; CM, cardiomyopathy; HD, hepatic disease; SGA, small-for-gestational-age; MRC, mitochondrial respiratory chain; C I, complex I; C II, complex II; C III, complex III; C IV, complex IV; OCR, oxygen consumption rate.
Disease types

Neonatal-onset

- MsMD: 23 (8%)
- CM: 26 (9%)
- LS: 58 (14%)
- HD: 194 (69%)

Characteristics

- Male to Female Ratio: 1:1 (138:143)
- Gestational age: 37±3 weeks (25w2d～41w4d)
- Birth weight: 2,346 ± 801g (422～4168 g)
- Premature delivery: 35.5%
- SGA: 35.3%

Hyperlactatemia

- 90% MsMD
- 80% CM
- 70% LS
- 76% HD

Initial symptoms by day of onset (321 symptoms / 281 patients)

- 236 (73.5%) patients developed symptoms within the first two days after birth.
Molecular diagnosis

Nuclear genes

Mitochondrial genes

Cox proportional hazards regression analyses

(A) LS vs non LS

(B) With vs without molecular diagnosis
DISCUSSIONS

- Preterm birth and SGA accounted for about 30% of the cases, which is similar to previous reports.\(^1,2\)
- Disease onset within the first 2 days of birth was 74%, with is higher than that in previous studies (51-59%).\(^1,2\) This is thought to be due to the fact that MD is now being diagnosed when a patient’s general condition deteriorates immediately after birth and early death occurs.
- Neonatal asphyxia of an unknown cause and sudden respiratory distress are common immediately after birth, and poor feeding, failure to thrive, drowsiness or poor health condition are common 2 days after birth, as previously reported.\(^1\)
- With respect to biochemical findings, hyperlactatemia was observed in 86% of patients, as in previous reports.\(^2\) It should be noted that the frequency is lower in organ-specific disease types such as CM.
- One of the reasons for the better prognosis of Leigh syndrome than other disease types is that there were fewer deaths in the neonatal period.

![Mortality in the neonatal period](image)

- The poor prognosis of patients with a molecular diagnosis suggests that patients with a mitochondrial dysfunction based on a biochemical analysis may include secondary mitochondrial dysfunctions other than MD.
- Among the patients diagnosed using the biochemical approach and with a confirmed molecular diagnosis, 18% (n=13 / 71) did not have decreased MRC enzyme activity and were screened for decreased OCR. The diagnosis of neonatalonset MD is based on a general method for diagnosing MD,\(^8\) but making a diagnosis is challenging because many patients die early during the clinical course, and the collection of samples during survival is difficult. If necessary, a combined analysis of MRC enzyme assay and OCR measurement will help confirm the diagnosis of neonatal-onset MD, which is difficult to diagnose clinically.
- On the contrary, depending on local accessibility to broad NGS approaches, diagnostic algorithms for MDs tend to favour genetic first over phenotype first.\(^4\) As we observed in a small number of patients, certain patients with possible MD cannot be diagnosed using biochemical analyses alone\(^9\) but could be diagnosed molecularly. However, biochemical tests are still valuable in critically ill patients for whom time is an issue and for functional validation of class 3 variants to establish a firm diagnosis. It is important to perform comprehensive testing by collecting appropriate tissue samples, while considering invasiveness, and by analysing the genes responsible.

CONCLUSIONS

- This observational study of neonatal-onset mitochondrial disease in the largest cohort ever is the first in Asia and shows more diverse genetic aetiology than that previously reported.
- Combining a mitochondrial respiratory chain enzyme assay with cellular oxygen consumption rate measurement increases the number of molecular diagnoses of neonatal-onset mitochondrial diseases.
- In neonatal-onset mitochondrial diseases, diagnosis of Leigh syndrome and confirmed molecular diagnosis have independent and significant effects on the survival curve.