SHORT REPORT

CLINICAL GENETICS WILEY

Prevalence and clinical prediction of mitochondrial disorders in a large neuropediatric cohort

Amelie T. van der Ven[1] | Jessika Johannsen[2] | Fanny Kortüm[1] |
Matias Wagner[3,4] | Konstantinos Tsiakas[2] | Tatjana Bierhals[1] |
Davor Lessel[1] | Theresia Herget[1] | Katja Kloth[1] | Jasmin Lisfeld[1] |
Tasja Scholz[1] | Nadia Obi[5] | Saskia Wortmann[3,4,6] | Holger Prokisch[3,4] |
Christian Kubisch[1,7] | Jonas Denecke[2] | René Santer[2,7] | Maja Hempel[1,7]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[2]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[3]Institute of Human Genetics, Klinikum Rechts der Isar, TUM, Munich, Germany

[4]Institute of Neurogenomics, Helmholtz Center Munich, Neuherberg, Germany

[5]Institute of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[6]Department of Pediatrics, University Medical Center Salzburg, Salzburg, Austria

[7]Martin Zeitz Center of Rare Diseases, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence
Maja Hempel, Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20251 Hamburg, Germany.
Email: maja.hempel@onlinehome.de

Abstract

Neurological symptoms are frequent and often a leading feature of childhood-onset mitochondrial disorders (MD) but the exact incidence of MD in unselected neuropediatric patients is unknown. Their early detection is desirable due to a potentially rapid clinical decline and the availability of management options. In 491 children with neurological symptoms, a comprehensive diagnostic work-up including exome sequencing was performed. The success rate in terms of a molecular genetic diagnosis within our cohort was 51%. Disease-causing variants in a mitochondria-associated gene were detected in 12% of solved cases. In order to facilitate the clinical identification of MDs within neuropediatric cohorts, we have created an easy-to-use bedside-tool, the MDC-NP. In our cohort, the MDC-NP predicted disease conditions related to MDs with a sensitivity of 0.83, and a specificity of 0.96.

KEYWORDS
child development disorders, early diagnosis, medical genetics, mitochondria, whole exome sequencing

1 | INTRODUCTION

With a prevalence of 11.5 per 100 000 in the general population, mitochondrial disorders (MDs) constitute the largest class of inborn

René Santer and Maja Hempel contributed equally to this work.

errors of metabolism.[1] The phenotypic presentation, especially of childhood-onset MDs, is extremely variable.[2] Approximately 45% of affected children come to medical attention because of neurologic symptoms.[2] The prevalence of MDs among neuropediatric patients, however, remains to be determined. Several sets of diagnostic criteria have been developed to facilitate the clinical recognition of MDs.[3-9] The most recent scoring system is the "revisited" MDC score[7] (Mitochondrial disease criteria, rMDC). The rMDC is based on a variety of anamnestic information and results of clinical investigations, which complicates its application in clinical practice. In addition, not all the items queried are suitable for a broad diagnostic work-up of neuropediatric cohorts (e.g., electromyography) or applicable to young children (e.g., exercise intolerance).

The first aim of our project was to systematically determine the prevalence of MDs in a heterogenous cohort of 491 neuropediatric patients. Our second concern was to develop a bed-side tool for quick and easy clinical identification of children with a high likelihood of underlying MDs within neuropediatric cohorts.

2 | METHODS

2.1 | Study design

From November 2014 to April 2020, a total of 491 unrelated children with undiagnosed neurological disorders were enrolled into a comprehensive diagnostic work-up including (Trio) exome sequencing (ES). See Supplementary Methods/Figure S1 for inclusion criteria, phenotyping, ES, and variant processing. ES results of 50 patients included here were previously described.[10] The study was approved by the ethics committee of the Hamburg Medical Association (PV3802/PV7038). Informed parental consent was mandatory for inclusion. All procedures adhered to the tenets of the Helsinki Declaration and its subsequent revisions.

2.2 | Genotype categorization

Genes identified to harbor a pathogenic, likely pathogenic or variant of uncertain significance (as per Reference [11]) were categorized into one of two groups: mitochondria-associated gene (MAG) or non-MAG. Genes were classified as MAG whenever mitochondrial relevance was indicated by at least three out of four databases (Human MitoCarta 2.0, "known mitochondrial" list: http://www.mrc-mbu.cam.ac.uk/, OMIM, GeneCards) and/or recently published as one of 338 mitochondrial disease genes.[12]

2.3 | Collection of clinical data and rMDC score

We performed a retrospective analysis of the medical reports by our genetics service, which were compiled immediately prior to initiation of ES for all 491 neuropediatric patients. Symptoms were indexed according to Human Phenotype Ontology (HPO). We then applied the rMDC to our cohort (Supplementary Methods, Tables S1A/S1B).

2.4 | Development of a simplified clinical evaluation tool

As a next step, we reviewed the rMDC results (Appendix S1: Supplementary Results) in context with the ES findings (i.e., disease-causing non-MAG variant vs. MAG variant identified). Again, considering our medical reports, we then composed a simplified clinical assessment tool tailored to the phenotypic characteristics of our neuropediatric cohort, referred to here as Mitochondrial Disease Criteria for Neuropediatric Patients (MDC-NP, Figure 1). In brief, most discriminative rMDC items (Table S4) were either directly included into the MDC-NP (e.g., ataxia; dystonia) or regrouped/combined for a facilitated score calculation process (e.g., lactate elevation in serum/CSF or peak in MRS). Additional items not explicitly listed in the rMDC score but frequently seen within our cohort of children with disease-causing MAG variants were also included in the MDC-NP. See Table S1C for detailed comparison of test items.

3 | RESULTS

3.1 | Phenotypic composition of the cohort

The spectrum of phenotypic presentations upon admission was very broad, involving a median of 6 out of 24 HPO major categories (range 1–14). See Supplementary Results for detailed phenotype information.
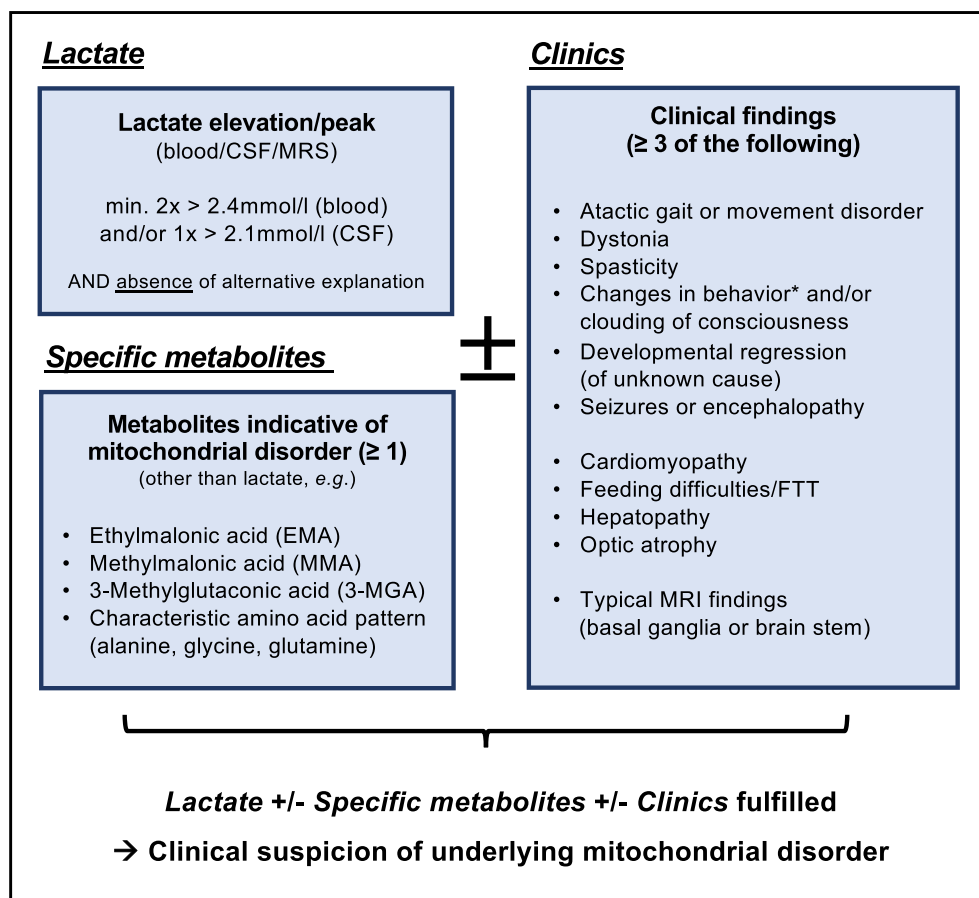
3.2 | Results of ES

In 250 out of 491 patients (51%), we identified a genetic cause of disease consisting of 231 single gene alterations and 19 CNV's. Of the 231 identified disease genes, 25 met our requirements for classification as MAG: *AARS1*, *BCS1L*, *DHTKD1*, *DHX30* (2×), *DNM1L*, *ETFDH*, *FASTKD2*, *GFM2*, *GLDC* (2×), *HSD17B10* (2×), *MPV17*, *MT-ND1*, *NDUFAF3*, *NDUFB11*, *NDUFB3*, *NDUFV1*, *NFU1*, *PDHA1*, *POLG*, *RARS2*, *SDHB*, *SERAC1*, *SLC19A3*, *SURF1* (2×), *TRMT5*. The remaining 206 genes were categorized as non-MAG.

Of the 250 patients with a molecular genetic diagnosis, 221 (88%) harbored a variant (including CNVs) in a non-MAG. In 29 patients (12%), variants affected MAGs (6% of total cohort) (Figure 2). Only one patient carried a variant in a mtDNA-located MAG (MT-ND1).

3.3 | Outcome: MDC-NP

The MDC-NP tool was applied to the entire cohort of 491 neuropediatric patients (Tables S6 and S7). An underlying MD was clinically

FIGURE 1   Clinical evaluation tool tailored to the phenotypic characteristics of our neuropediatric cohort (here referred to as Mitochondrial Disease Criteria for Neuropediatric Patients [MDC-NP]). Whenever one or more diagnostic category is met ("lactate", "specific metabolites" and/or "clinics"), a mitochondrial disorder is clinically suspected. CSF, cerebrospinal fluid; FTT, failure to thrive; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy. *Changes in behavior, for example, new onset of depression, psychosis, dementia [Colour figure can be viewed at wileyonlinelibrary.com]

Lactate

Lactate elevation/peak
(blood/CSF/MRS)

min. 2x > 2.4mmol/l (blood)
and/or 1x > 2.1mmol/l (CSF)

AND absence of alternative explanation

Specific metabolites

Metabolites indicative of mitochondrial disorder (≥ 1)
(other than lactate, e.g.)

- Ethylmalonic acid (EMA)
- Methylmalonic acid (MMA)
- 3-Methylglutaconic acid (3-MGA)
- Characteristic amino acid pattern (alanine, glycine, glutamine)

Clinics

**Clinical findings
(≥ 3 of the following)**

- Atactic gait or movement disorder
- Dystonia
- Spasticity
- Changes in behavior* and/or clouding of consciousness
- Developmental regression (of unknown cause)
- Seizures or encephalopathy

- Cardiomyopathy
- Feeding difficulties/FTT
- Hepatopathy
- Optic atrophy

- Typical MRI findings (basal ganglia or brain stem)

Lactate +/- Specific metabolites +/- Clinics fulfilled
→ Clinical suspicion of underlying mitochondrial disorder

suspected whenever one or more of the MDC-NP categories was met (Figure 1). In brief, the "lactate"-category was met whenever an elevated lactate concentration was detected in either serum, CSF and/or MRS. "Specific metabolites" was fulfilled whenever indicative biochemical parameters were present. "Clinics" comprises a list of suggestive clinical signs/symptoms and was considered fulfilled whenever ≥3 symptoms were present.

Within our total cohort of 491 patients, 49 patients (10%) met MDC-NP criteria. From our cohort with a molecular genetic diagnosis (250 patients), 32 patients fulfilled MDC-NP criteria (13%). In 24 out of 32 patients (75%) who met MDC-NP criteria, a disease-causing MAG variant was identified (Figure S3A/Table S5A). Five patients who did not fulfill MDC-NP criteria were found to actually have a causative MAG variant (Figure S3B/Table S5B). For eight patients with suspected MD based on MDC-NP, a non-MAG variant was detected (Figure S3C/Table S5C). In summary, the MDC-NP predicted MDs with a specificity of 0.96 (CI 0.93–0.98) and a sensitivity of 0.83 (CI 0.65–0.93) (Supplementary Methods for Statistics, Figure 3, Figures S4/S5).

4 | DISCUSSION

In our neuropediatric cohort, (trio-)ES was able to detect a disease-causing MAG variant in 12% of the 250 cases with a molecular

genetic diagnosis. Thus, our results confirm that MDs are a significant cause of disease in neuropediatric patients. The identified MAG variants were predominantly located on the nDNA. This finding was expected as nDNA-variants are the major cause of early childhood onset MDs.[7,13]

An early identification of MDs is important in the context of clinical management, and sensitive and easy to use diagnostic tools for rapid clinical diagnosis remain necessary. In our experience, the rMDC is too comprehensive to be easily integrated into routine clinical practice. Additional limitations identified here include: (i) young children with unspecific/oligosymptomatic MD-manifestations do not necessarily meet the high-diagnostic threshold required by the rMDC (Table S5B); (ii) clinical items included in the rMDC sometimes have little/no discriminatory value in neuropediatric patients (Table S4). To address these concerns, we selected readily available features that most consistently differed between our patients with disease-causing variants in MAGs versus non-MAGs (Table S7) and compiled them into a simplified clinical prediction tool, which we here refer to as MDC-NP (Figure 1).

When applied to our heterogenous neuropediatric cohort, the MDC-NP tool clinically predicted an MD with a sensitivity of 0.83 (CI 0.65–0.93) and a specificity of 0.96 (CI 0.93–0.98), whereas the rMDC had a sensitivity of 0.59 (CI 0.41–0.75) and a specificity of 0.99 (CI 0.96–1.00) (Supplementary Results, Figures S4 and S5). In our opinion, it is time to shift from predominantly highly specific clinical

Genotype composition total cohort (n=491)
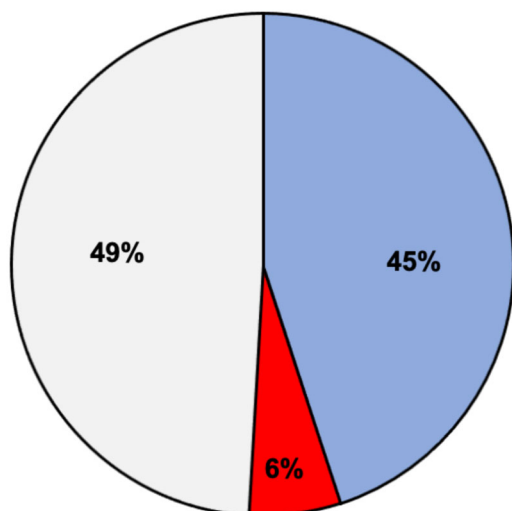
- □ Non-mitochondria-associated gene (n=221)
- ■ Mitochondria-associated gene (n=29)
- □ Unsolved or finding in candidate gene (n=241)

FIGURE 2 Results of genotyping (total cohort $n = 491$). In 250 patients (51% of total cohort), we identified a pathogenic variant in an established disease gene. Of these patients with a molecular genetic diagnosis, 221 (45% of total cohort) had findings in known non-mitochondria-associated genes or CNVs. Another 29 patients had disease-causing variants in mitochondria-associated genes (6% of total cohort). The remaining 49% of the total cohort (241 patients) consists of either unsolved cases (139 patients), or patients in whom a putative disease-causing variant in a candidate disease gene was identified (103 patients) [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Predictive value of MDC-NP. In 491 neuropediatric patients, a comprehensive diagnostic work-up was performed. In 250 of the 491 patients (51%), (trio-)ES could establish the underlying molecular diagnosis. MDC-NP was applied to the total cohort, only the results of the 250 patients with a molecular genetic diagnosis are presented here. From these 250 patients, 32 individuals met MDC-NP criteria (13%). In 24 of these 32 patients, the disease-causing variant was located within a mitochondria-associated gene. The MDC-NP yielded false negative results in 5 patients (i.e., clinically missed an underlying mitochondrial disorder) [Colour figure can be viewed at wileyonlinelibrary.com]

assessment scales toward screening tools with higher sensitivity as the diagnostic work-flow applied to suspected MDs has also shifted away from the "biopsy-first" toward a "genetic-first" approach.[9] Patients identified at risk can benefit from the positive consequences of an early diagnosis (rapid molecular diagnostics, clinical management adaptation), without the fear of invasive procedures.

MD genes are not unequivocally defined, and the pathophysiological understanding of MDs is subject to ongoing adjustments.[12,14] To account for these dynamics, we have used here the mitochondrial relevance of genes as indicated by publicly available databases (i.e., MAG, see Section 2) as a straightforward and easily objectifiable approximation to MD genes. We recognize, that the sensitivity and specificity values calculated here are influenced by this approach. *DHX30* is particularly notable for its debatable subcategorization as MAG because the mitochondrial role of *DHX30* is ultimately attributed to only a single transcript isoform.[15] Significantly, both MDC-NP and rMDC revealed no clinical suspicion of an underlying MD in our two *DHX30*-patients (Table S5B). In contrast, the specificity values of both the MDC-NP and the rMDC are equally affected by our genetic categorization of *SLC52A3* and *TANGO2* as non-MAG. While not
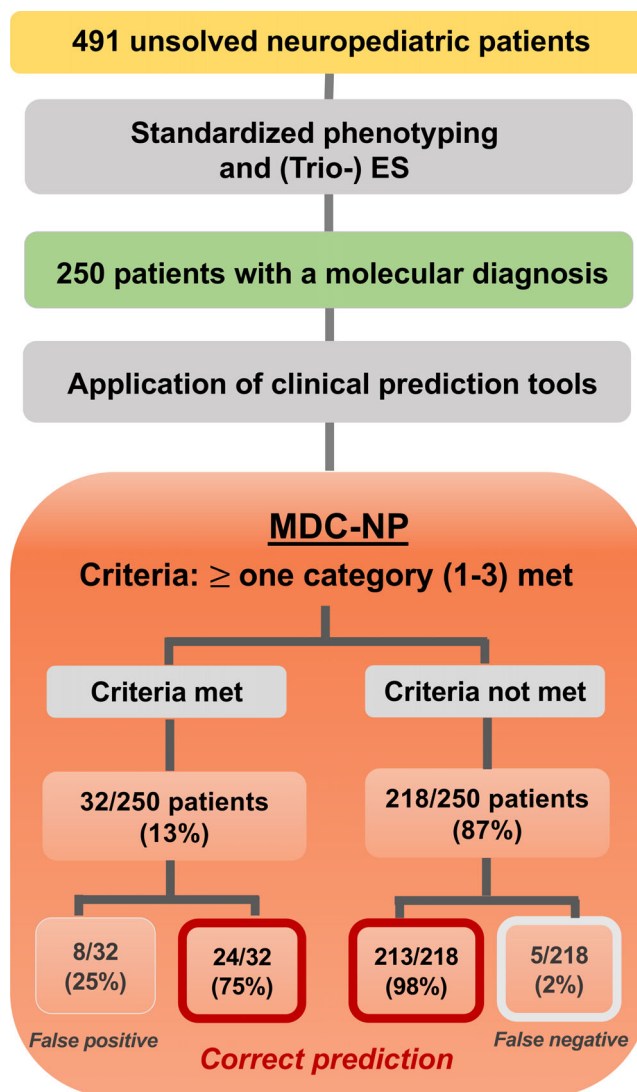
being localized within the mitochondria themselves, both genes exhibit an established secondary effect on mitochondria.[16,17] Our *SLC52A3-/TANGO2*-patients were consequentially clinically predicted to have an MD (Table S5C).

While these two examples may challenge our genetic subclassification approach, they concurrently accentuate the predictive qualities of the MDC-NP-score. Overall, we believe that our criteria are sufficient to reliably assign the vast majority of the 231 disease-genes identified here.

We realize, that also the items included in the MDC-NP leave room for further improvement. Just as previously published rating scales for the clinical diagnosis of MDs,[3-8,18] the MDC-NP in its current state is based on a retrospective analysis of patients, and future prospective analyses in independent cohorts are certainly required for validation of the results presented here.

5 | CONCLUSION

Our data demonstrate that mitochondrial disorders are present in a substantial proportion of neuropediatric patients. We propose the MDC-NP as an easy-to-use bedside screening tool specifically tailored to the clinical identification of neuropediatric patients with a high likelihood of an underlying mitochondrial disorder.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14061.

DATA AVAILABILITY STATEMENT

The majority of data generated or analyzed during this study are included in this published article [and the supporting information files]. The remaining data are available from the corresponding author upon request.

ORCID

Amelie T. van der Ven https://orcid.org/0000-0002-4167-8411
Matias Wagner https://orcid.org/0000-0002-4454-8823
Davor Lessel https://orcid.org/0000-0003-4496-244X
Maja Hempel https://orcid.org/0000-0003-0609-2738

REFERENCES

1. Chinnery PF. Mitochondrial disorders overview; 2014.
2. Koenig MK. Presentation and diagnosis of mitochondrial disorders in children. *Pediatr Neurol*. 2008;38(5):305-313.
3. Walker UA, Collins S, Byrne E. Respiratory chain encephalomyopathies: a diagnostic classification. *Eur Neurol*. 1996; 36(5):260-267.
4. Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology*. 2002;59(9):1406-1411.
5. Thorburn DR, Smeitink J. Diagnosis of mitochondrial disorders: clinical and biochemical approach. *J Inherit Metab Dis*. 2001;24(2): 312-316.
6. Wolf NI, Smeitink JA. Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children. *Neurology*. 2002;59(9): 1402-1405.
7. Witters P, Saada A, Honzik T, et al. Revisiting mitochondrial diagnostic criteria in the new era of genomics. *Genet Med*. 2018;20(4): 444-451.
8. Joost K, Rodenburg RJ, Piirsoo A, et al. A diagnostic algorithm for mitochondrial disorders in Estonian children. *Mol Syndromol*. 2012; 3(3):113-119.
9. Wortmann SB, Mayr JA, Nuoffer JM, Prokisch H, Sperl W. A guideline for the diagnosis of pediatric mitochondrial disease: the value of muscle and skin biopsies in the genetics era. *Neuropediatrics*. 2017;48(4): 309-314.
10. Mahler EA, Johannsen J, Tsiakas K, et al. Exome sequencing in children. *Dtsch Arztebl Int*. 2019;116(12):197-204.
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5): 405-424.
12. Stenton SL, Prokisch H. Genetics of mitochondrial diseases: identifying mutations to help diagnosis. *EBioMedicine*. 2020;56: 102784.
13. Barca E, Long Y, Cooley V, et al. Mitochondrial diseases in North America: an analysis of the NAMDC registry. *Neurol Genet*. 2020;6(2): e402.
14. Rodenburg RJ. Mitochondrial complex I-linked disease. *Biochim Biophys Acta*. 2016;1857(7):938-945.
15. Lessel D, Schob C, Kury S, et al. De novo missense mutations in DHX30 impair global translation and cause a neurodevelopmental disorder. *Am J Hum Genet*. 2018;102(1):196.
16. Udhayabanu T, Manole A, Rajeshwari M, Varalakshmi P, Houlden H, Ashokkumar B. Riboflavin responsive mitochondrial dysfunction in neurodegenerative diseases. *J Clin Med*. 2017;6(5):52.
17. Jennions E, Hedberg-Oldfors C, Berglund AK, et al. TANGO2 deficiency as a cause of neurodevelopmental delay with indirect effects on mitochondrial energy metabolism. *J Inherit Metab Dis*. 2019;42(5): 898-908.
18. Morava E, van den Heuvel L, Hol F, et al. Mitochondrial disease criteria: diagnostic applications in children. *Neurology*. 2006;67(10): 1823-1826.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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