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

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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...
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” (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 heterogeneous cohort of 491 neuropediatric patients. Our second concern was to develop a bedside tool for quick and easy clinical identification of children with a high likelihood of underlying MDs within neuropediatric cohorts.

## METHODS

### 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.

### 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.\(^12\)

### 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).

### 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.

## RESULTS

### 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.

### 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 CNVs. Of the 231 identified disease genes, 25 met our requirements for classification as MAG: AARS1, BCS1L, DHTKD1, DHX30 (2\(\times\)), DNLM1L, ETFDH, FASTKD2, GFM2, GLDC (2\(\times\)), HSD17B10 (2\(\times\)), MPV17, MT-ND1, NDUFV3, NDUFB11, NDUFB3, NDUVF1, NFU1, PDHA1, POLG, RARS2, SDHB, SERAC1, SLC19A3, SURF1 (2\(\times\)), 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).

### 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
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
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. 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. 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. 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. 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.

![Diagram](image-url)
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, 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.

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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.

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SUPPORTING INFORMATION

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

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