Mitochondrial DNA Mutations and Pediatric Neurological Disorders: A Systematic Review

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Abstract

Background:

Mitochondrial DNA mutations are increasingly recognized as a significant cause of neurological disease in the pediatric population, yet the breadth of genotype–phenotype associations, diagnostic yield, and clinical spectrum across different study designs and populations have not been systematically synthesized.

Methods:

A systematic search of PubMed, EMBASE, and Scopus was conducted from inception until 10 May 2026 to identify studies evaluating mitochondrial DNA mutations in pediatric patients with neurological or neurodevelopmental disorders. All authors independently performed study selection; discrepancies were resolved through discussion. Data extraction and risk-of-bias assessment were performed using standardized tools adapted for observational study designs. Narrative synthesis was conducted.

Result:

Twenty-one studies met inclusion criteria, encompassing cohort, case-control, and diagnostic studies from twelve countries. Leigh syndrome and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes were the most frequently reported phenotypes. Recurrent mutations in MT-ATP6, MT-TL1, MT-ND3, MT-ND5, and MT-ND6 were identified across studies. Autism spectrum disorder studies yielded mixed results regarding mitochondrial DNA involvement. Overall risk of bias ranged from low–moderate to high.

Conclusions:

Mitochondrial DNA mutations contribute to a wide spectrum of pediatric neurological disease, with Leigh syndrome and mitochondrial encephalomyopathy predominating. Standardized phenotyping and comprehensive sequencing approaches are needed for future multicenter studies.

Introduction

Mitochondrial diseases represent a clinically heterogeneous group of inherited metabolic disorders arising from dysfunction of the mitochondrial oxidative phosphorylation system, with an estimated combined prevalence of approximately 1 in 5,000 live births, making them among the most common inborn errors of metabolism encountered in pediatric clinical practice.[1,2] The mitochondrial genome, a 16,569-base-pair circular double-stranded DNA molecule encoding 13 essential subunits of the respiratory chain complexes, 22 transfer ribonucleic acids, and 2 ribosomal ribonucleic acids, is maternally inherited and present in hundreds to thousands of copies per cell, giving rise to the phenomenon of heteroplasmy whereby mutant and wild-type mitochondrial DNA molecules coexist within individual cells and tissues.[3] The nervous system, by virtue of its extraordinary metabolic demands and near-complete dependence on oxidative phosphorylation for adenosine triphosphate generation, is disproportionately vulnerable to mitochondrial dysfunction, and neurological manifestations including epilepsy, encephalopathy, stroke-like episodes, developmental regression, optic neuropathy, and movement disorders constitute the most frequent and debilitating clinical presentations of mitochondrial DNA-related disease in childhood.[4,5]

Over the past two decades, advances in molecular diagnostic technologies, from targeted polymerase chain reaction and restriction fragment length polymorphism analysis to next-generation sequencing and whole-genome approaches, have substantially expanded the catalog of pathogenic mitochondrial DNA mutations associated with pediatric neurological disease and have enabled the identification of novel variants, the quantification of tissue-specific heteroplasmy, and the delineation of genotype–phenotype correlations with increasing precision.[6,7] Classic mitochondrial DNA-associated neurological syndromes such as Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes, myoclonic epilepsy with ragged red fibers, and Leber hereditary optic neuropathy have been extensively characterized in individual cohort studies; however, the evidence base is fragmented across diverse populations, study designs, molecular platforms, and phenotypic classification systems, and no systematic synthesis has comprehensively collated the preclinical and clinical evidence regarding the spectrum, frequency, and clinical significance of mitochondrial DNA mutations in pediatric neurological and neurodevelopmental disorders.[8–10] Furthermore, a growing body of literature has explored the potential contribution of mitochondrial DNA variation, including deletions, copy-number alterations, and haplogroup associations, to neurodevelopmental conditions such as autism spectrum disorder, but the evidence remains conflicting and has not been systematically appraised.[11,12]

A systematic review synthesizing the available evidence on mitochondrial DNA mutations in pediatric neurological disorders is warranted to consolidate the current state of knowledge, characterize the range of mitochondrial genotypes and neurological phenotypes reported, evaluate the methodological rigor of existing studies, and identify gaps that should inform future research priorities including standardized phenotyping, comprehensive sequencing strategies, and multinational collaborative investigations. Therefore, the objective of this systematic review was to systematically identify, critically appraise, and narratively synthesize studies evaluating the association between mitochondrial DNA mutations and neurological or neurodevelopmental disorders in the pediatric population.

Materials and method

Search Strategy

A comprehensive systematic literature search was performed across three electronic databases, which are PubMed, EMBASE, and Scopus, from inception until 10 May 2026. The search strategy combined Medical Subject Headings and free-text terms across three conceptual

domains: mitochondrial DNA ("mitochondrial DNA" OR "mtDNA" OR "mitochondrial genome" OR "mitochondrial mutation" OR "mitochondrial deletion" OR "heteroplasmy" OR "mtDNA copy number"), pediatric population ("pediatric" OR "paediatric" OR "child" OR "children" OR "infant" OR "neonatal" OR "adolescent"), and neurological disorders ("neurological" OR "encephalopathy" OR "epilepsy" OR "Leigh syndrome" OR "MELAS" OR "autism" OR "neurodevelopmental" OR "demyelination" OR "optic neuropathy"). Boolean operators combined domains with AND logic. Reference lists of retrieved articles were manually screened to identify additional eligible studies. All authors independently performed study selection at the title/abstract and full-text screening stages, and any discrepancies were resolved through consensus discussion.

Eligibility Criteria

Studies were selected according to predefined eligibility criteria structured using the Population, Exposure, Comparator, and Outcome framework. The population comprised human pediatric patients (preferably aged 0–18 years) with neurological or neurodevelopmental disorders or suspected/confirmed mitochondrial disease with neurological manifestations. The exposure (index finding) was defined as mitochondrial DNA mutations or variants, including point mutations, single large-scale deletions, heteroplasmy assessments, haplogroup associations, mitochondrial DNA copy-number alterations, and mitochondrial DNA–encoded respiratory chain gene variants. Comparators included healthy controls, disease controls, mitochondrial DNA–negative patients, nuclear DNA mutation groups, or within-cohort genotype–phenotype comparisons. Outcomes of interest included neurological diagnosis and phenotype, epilepsy and seizures, encephalopathy, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes features, demyelination, autism and neurodevelopmental phenotype, neuroimaging findings, disease severity, progression, mortality, genotype–phenotype correlation, and diagnostic yield. Eligible study designs included cohort, case-control, randomized controlled trials, and diagnostic cross-sectional studies with controls. Reviews, protocols, conference abstracts, animal studies, in vitro–only studies, and case reports or very small non-comparative reports were excluded. The search was restricted to English-language publications with no time-frame limitation.

Data Extraction

Data were extracted independently by all authors using a standardized electronic workbook. The following variables were recorded for each included study: first author and publication year, country, study design, population and clinical setting, sample size, mitochondrial DNA exposure or testing method, comparator group, neurological outcomes and phenotypes, key findings, and eligibility notes. Discrepancies in data extraction were resolved through discussion among the authors.

Risk of Bias Assessment

Risk of bias was assessed using domains adapted from the Risk of Bias in Non-Randomized Studies of Interventions tool and the Newcastle–Ottawa Scale, as appropriate for each study design. The following domains were evaluated: selection and participant recruitment, exposure assessment, outcome assessment, confounding and comparability, and missing data and selective reporting. Each domain was judged qualitatively, and an overall risk-of-bias judgement (low, low–moderate, moderate, moderate–high, or high) was assigned for each study based on the aggregate domain-level assessments.

Results

Study Selection

A total of 581 records were identified from PubMed (n = 214), EMBASE (n = 177), and Scopus (n = 190). After removing duplicate records (n = 43), 538 records were screened, of which 490 were

excluded. Subsequently, 48 reports were sought for retrieval, and all were successfully retrieved. These 48 reports were assessed for eligibility, with 27 reports excluded for the following reasons: narrative reviews, scoping reviews, systematic reviews, and meta-analyses (n = 4); mixed-age studies in which pediatric subgroup data aged 0–18 years were not separately reported or extractable (n = 7); and studies on mitochondrial dysfunction without molecular characterization of mtDNA (n = 16). Finally, 21 studies were included in the review (figure 1).

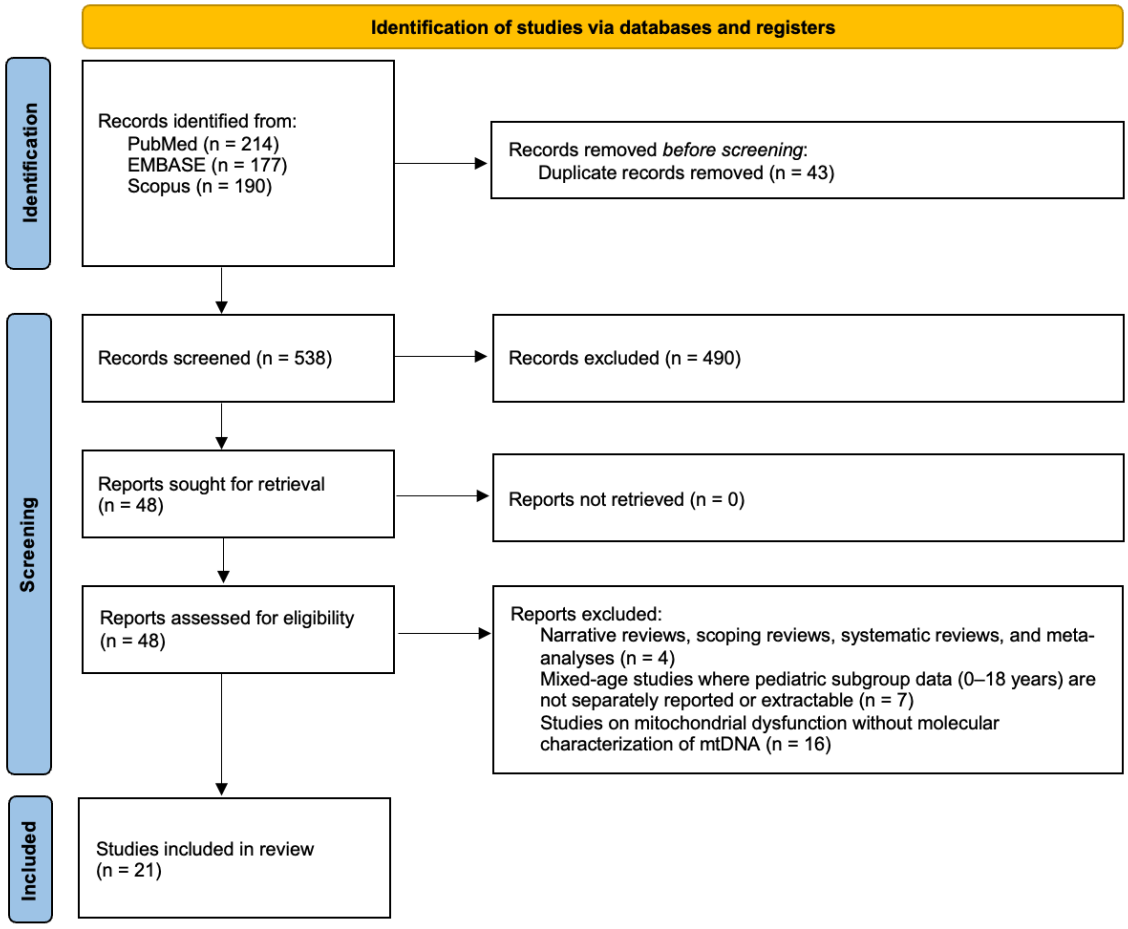

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram illustrating the study selection process.

Study and Baseline Characteristics

The twenty-one included studies were published between 2000 and 2026, originating from twelve countries across Europe, East Asia, the Middle East, Africa, North America, South America, and Oceania (Table 1). Study designs comprised retrospective cohorts (twelve studies), case-control studies (five studies), diagnostic/cross-sectional cohorts (three studies), and a population-based family cohort (one study). Sample sizes ranged from 13 to 1,298 cases. The clinical populations encompassed pediatric Leigh syndrome cohorts (five studies), mixed pediatric mitochondrial disease cohorts (seven studies), pediatric acquired demyelinating syndromes (one study), and autism spectrum disorder cohorts (six studies). Mitochondrial DNA testing methods included targeted polymerase chain reaction and restriction fragment length polymorphism screening, Sanger sequencing, whole mitochondrial genome sequencing, next-generation sequencing panels, whole-exome sequencing with mitochondrial DNA coverage, and genome-

wide mitochondrial single nucleotide polymorphism arrays. The most frequently reported mitochondrial DNA mutations across studies were MT-ATP6 m.8993T>G and m.8993T>C, MT-TL1 m.3243A>G, MT-TK m.8344A>G, and MT-ND3/MT-ND5/MT-ND6 complex I subunit mutations. Leigh syndrome and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes were the predominant neurological phenotypes. Six studies focused on autism spectrum disorder and mitochondrial DNA variation, yielding mixed results. The characteristics of included studies are presented in Tables 1 and 2.

Risk of Bias

The risk-of-bias assessment for the twenty-one included studies is summarized in Table 3. Overall risk of bias was judged as moderate in thirteen studies, moderate to high in five studies, low to moderate in two studies, and high in one study. The most common sources of bias included referral-based or tertiary-center recruitment leading to selection bias, the absence of external comparator groups in many cohort studies, retrospective data abstraction with incomplete outcome ascertainment, and variable molecular testing methods across and within studies. Exposure assessment was generally rated favorably when studies employed comprehensive mitochondrial DNA sequencing, but was rated less favorably in studies using targeted mutation panels that may underestimate the true frequency of pathogenic variants. Confounding and comparability were particular concerns in case-control autism spectrum disorder studies, where age and sex mismatches between cases and controls were identified. The domain-level risk-of-bias results are presented in Table 3.

Table 1. Characteristics of included studies.

Author/Year	Country	Population	N (cases)	mtDNA Test	Key mtDNA Findings
Ardissone 2023	Italy	Pediatric mtDNA disease	150	PCR/Sanger/NGS	LS 36%, LHON 17%, MELAS 11%
Liu 2025	China	Suspected mito disorder	227 mtDNA+	WES + whole mtDNA	5 novel pathogenic variants
Yang 2026	China	Suspected mito disorder	47 confirmed	NGS/WES/mtDNA	MELAS 45%, LS 28%
Mehaney 2024	Egypt	Suspected mito syndromes	114 + 50 ctrl	PCR/RFLP panel	1.8% mtDNA positive
Lee 2019	S. Korea	mtDNA-assoc LS	25	Whole mtDNA seq	Epilepsy 56%
Lee 2016	S. Korea	Leigh syndrome	39 (11 mtDNA+)	Targeted mtDNA	MT-ND genes 82%
Yu 2018	China	mtDNA-positive LS	13	Whole mtDNA seq	MT-ND 62%, MT-ATP6 31%
Naess 2009	Sweden	Pediatric LS	25 (8 mtDNA+)	Whole mtDNA seq	32% mtDNA mutations
Makino 2000	Japan	Clinical LS	100 (18 mtDNA+)	Targeted ATPase 6	18% ATPase 6 mutations
Uusimaa 2007	Finland	m.3243A>G carriers	18 children	ApaI RFLP	Prev 18.4/100,000
Venkateswaran 2011	Canada	Ped demyelinating	213 + 166 ctrl	31 mtDNA SNPs	13708A OR 2.27
Danhelovska 2020	Czech Rep	MT-ND disease	13	mtDNA seq + RFLP	MT-ND5 69%
Schoonen 2019	S. Africa	Pediatric MD	212 (123 seq)	Whole mtDNA + NGS	No causal mtDNA variant
Riley 2020	Australia	Suspected MD	40 trios	Genome sequencing	4 mtDNA diagnoses
Tolomeo 2021	Italy	Suspected MD	111 (82 seq)	Whole mtDNA + panel	8 mtDNA point mutations
Wong 2016	USA	Autism vs TD	66 AU + 46 TD	qPCR CN/deletion	mtDNA del OR 3.1
Varga 2018	Hungary	ASD vs controls	60 ASD + 60 ctrl	Long-range PCR	mtDNA del OR 5.8
Adiba 2024	Bangladesh	Autism vs controls	50 ASD + 30 ctrl	Sanger Cytb region	57 variants; 4 severity-assoc
Alvarez-Iglesias 2011	Spain	ASD vs controls	148 ASD + 753 ctrl	Minisequencing 25 mut	No pathogenic mut found
Hadjixenofontos 2013	USA	ASD vs controls	1298 + 2646 ctrl	SNP/haplo/reseq	No mtDNA association
Weissman 2008	USA	ASD + mito disease	25	ETC + partial mtDNA	2 likely pathogenic variants

Retro, retrospective; LS, Leigh syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; LHON, Leber hereditary optic neuropathy; MD, mitochondrial disease; ASD, autism spectrum disorder; AU, autism; TD, typically developing; CC, case-control; seq, sequencing; mut, mutations; del, deletion; CN, copy number; ctrl, controls; Pop, population; Prev, prevalence; OR, odds ratio.

Table 2. Key neurological outcomes of included studies.

Author/Year	Neurological Outcomes	Key Findings
Ardissone 2023	LS, LHON, MELAS, KSS/PEO, MERRF phenotyping	LS 36% with onset 18 mo; deep gray matter and early onset = poor prognosis
Liu 2025	LS, MELAS, CPEO, MERRF phenotypes	54 pathogenic variants in 227 children; 5 novel variants functionally verified
Yang 2026	MELAS, LS, epilepsy, stroke-like episodes	Epilepsy 64.5% mtDNA group; stroke-like episodes 58.1%; 6 fatal cases showed neuroimaging–metabolic–cardiac triad
Mehaney 2024	LHON, MELAS, NARP/LS, MERRF screening	Only 2/114 positive (both LHON m.14484T>C); 67.5% consanguinity suggests nuclear origin
Lee 2019	Epilepsy in mtDNA-LS; EEG, MRI/MRS	Epilepsy 56%; diffuse atrophy (64.3% vs 18.2%, p=0.042) and GI symptoms more frequent with epilepsy
Lee 2016	LS neurologic progression, functional outcome	Deterioration 74%; onset <1 yr and extra-BG MRI lesions predict poor outcome
Yu 2018	LS clinical/neuroimaging/pathology	Brainstem involvement 12/13; no epileptic seizures; CSF lactate elevated in all tested
Naess 2009	LS seizures, lactate, survival	Seizures 64%; mortality 68%; no clear mtDNA vs nuclear prognostic difference
Makino 2000	LS onset/course by ATPase 6 mutation	T8993G: early onset, rapid deterioration; T8993C: milder; T9176C: variable
Uusimaa 2007	m.3243A>G childhood phenotype	Hearing loss, short stature, migraine, learning difficulties; 5/24 abnormal EEG
Venkateswaran 2011	PD-ADS, MS conversion, haplogroups	13708A OR 2.27 for PD-ADS; haplogroup H OR 2.60 for MS; no LHON mutations
Danhelovska 2020	LS/MELAS/overlap; MRI, prognosis	Early onset and higher heteroplasmy = worse prognosis; novel m.13091T>C
Schoonen 2019	Pediatric MD diagnostic yield	No causal mtDNA variant in 123 sequenced; muscle involvement 73%; African cohort underserved
Riley 2020	MD diagnostic utility of GS	67.5% likely causal variant; 4 mtDNA diagnoses; heteroplasmy 40–100% in blood
Tolomeo 2021	MD diagnosis, epilepsy, MRI predictors	BG involvement predicted mtDNA disease (p=0.008); 8 mtDNA point mutations
Wong 2016	Autism; mtDNA CN and deletions	mtDNA CN higher in AU (p=.012); deletions OR 3.1; paternal deletion signal
Varga 2018	ASD; mtDNA deletions	Deletions 16.6% ASD vs 3.3% control (OR 5.8, p=0.03); no hotspot mutations
Adiba 2024	Autism; Cytb variants, CN, biochemistry	4 severity-associated variants (OR 11.21); elevated lactate/ammonia in ASD
Alvarez-Iglesias 2011	ASD; mtDNA mutations/haplogroups	All ASD wild-type for 25 mutations; no haplogroup association after correction
Hadjixenofontos 2013	ASD; mtDNA SNPs/haplogroups/reseq	No evidence for major mtDNA role; negative across all three phases
Weissman 2008	ASD + mito disease; ETC, mtDNA variants	Complex I deficiency 64%; 2 likely pathogenic mtDNA variants

LS, Leigh syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; LHON, Leber hereditary optic neuropathy; MERRF, myoclonic epilepsy with ragged red fibers; KSS, Kearns–Sayre syndrome; PEO, progressive external ophthalmoplegia; NARP, neuropathy, ataxia and retinitis pigmentosa; BG, basal ganglia; EEG, electroencephalogram; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; PD-ADS, pediatric acquired demyelinating syndrome; MS, multiple sclerosis; MD, mitochondrial disease; GS, genome sequencing; CN, copy number; AU, autism; ASD, autism spectrum disorder; ETC, electron transport chain; GI, gastrointestinal; OR, odds ratio.

Table 3. Risk-of-bias assessment of included studies.

Author/Year	Selection	Exposure	Outcome	Confounding	Missing Data	Overall
Ardissone 2023	SC	L	SC	SC	SC	Moderate
Liu 2025	SC	L	SC	SC	SC	Moderate
Yang 2026	SC	L	SC	SC	SC	Moderate
Mehaney 2024	SC	SC	SC	L	SC	Mod–High
Lee 2019	SC	L	L	SC	SC	Moderate
Lee 2016	SC	SC	SC	SC	SC	Moderate
Yu 2018	SC	L	L	SC	SC	Mod–High
Naess 2009	SC	L	SC	SC	SC	Moderate
Makino 2000	SC	SC	SC	SC	H	Mod–High
Uusimaa 2007	L	L	L	SC	SC	Moderate
Venkateswaran 2011	L	SC	L	SC	SC	Moderate
Danhelovska 2020	SC	L	SC	SC	SC	Mod–High
Schoonen 2019	SC	L	SC	SC	SC	Moderate
Riley 2020	L	L	L	SC	SC	Low–Mod
Tolomeo 2021	SC	L	SC	SC	SC	Moderate
Wong 2016	L	SC	L	SC	SC	Moderate
Varga 2018	SC	SC	L	H	SC	Mod–High
Adiba 2024	SC	SC	SC	SC	SC	Mod–High
Alvarez-Iglesias 2011	L	SC	L	L	SC	Moderate
Hadjixenofontos 2013	L	L	L	SC	SC	Low–Mod
Weissman 2008	H	SC	SC	H	SC	High

L, low risk; SC, some concerns; H, high risk; Mod, moderate. Risk-of-bias domains adapted from ROBINS-I and Newcastle–Ottawa Scale as appropriate for study design.

Discussion

This systematic review identified twenty-one studies evaluating the association between mitochondrial DNA mutations and neurological or neurodevelopmental disorders in the pediatric population, encompassing a diverse range of study designs, populations, and molecular diagnostic approaches across twelve countries. The findings demonstrate that mitochondrial DNA mutations contribute to a broad spectrum of pediatric neurological disease, with Leigh syndrome and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes representing the most frequently reported clinical phenotypes.[13–33] Recurrent pathogenic mutations in MT-ATP6 (m.8993T>G, m.8993T>C, m.9176T>C), MT-TL1 (m.3243A>G), MT-TK (m.8344A>G), and complex I subunit genes (MT-ND3, MT-ND5, MT-ND6) were identified across multiple independent cohorts, affirming the clinical relevance of these genetic loci in pediatric mitochondrial neurology. Diagnostic yields for mitochondrial DNA testing varied substantially across studies, ranging from 1.8% in a targeted panel screening study to 100% in selected mitochondrial DNA–positive cohorts, underscoring the influence of patient selection, pre-test probability, and molecular testing methodology on ascertainment rates. The six autism spectrum disorder studies yielded conflicting results: two case-control studies reported significantly elevated mitochondrial DNA deletion frequencies in affected children, while the two largest genetic association studies found no evidence for a major mitochondrial DNA contribution to autism susceptibility after appropriate statistical correction.

The present findings are consistent with and extend the established literature on mitochondrial DNA-related disease in childhood. The predominance of Leigh syndrome among the included cohorts aligns with prior epidemiological data indicating that Leigh syndrome is the single most common pediatric presentation of mitochondrial disease, affecting approximately one-third to one-half of children with confirmed mitochondrial DNA mutations.[34,35] The recurrent identification of MT-ATP6 m.8993T>G as a major cause of Leigh syndrome across Japanese, Korean, Italian, Swedish, and Chinese cohorts in this review corroborates the known worldwide distribution of this mutation and its established association with severe early-onset neurodegeneration.[13,17,21,22] The observation by Mehaney and colleagues that only 1.8% of Egyptian children with clinically suspected mitochondrial syndromes harbored detectable mitochondrial DNA point mutations, despite a targeted panel approach, suggests that nuclear DNA mutations may predominate in consanguineous populations and highlights the importance of comprehensive genomic testing strategies.[16] The finding by Riley and colleagues that genome sequencing achieved a diagnostic yield of 67.5% in Australian children with suspected mitochondrial disease, including four mitochondrial DNA diagnoses, supports the growing consensus that whole-genome or exome-plus-mitochondrial-genome approaches should supersede targeted panels as the first-line molecular diagnostic strategy in pediatric mitochondrial disease.[26] With regard to autism spectrum disorder, the negative findings from the large multi-phase study by Hadjixenofontos and colleagues and the targeted mutation screening by Alvarez-Iglesias and colleagues provide robust evidence against a major role for common mitochondrial DNA variants in autism susceptibility at the population level, while the positive deletion findings of Wong and colleagues and Varga and colleagues suggest that mitochondrial DNA instability may characterize a subgroup of affected children, consistent with the concept of mitochondrial dysfunction as a secondary or modulatory factor rather than a primary etiological mechanism.[28–33]

The central nervous system represents the tissue with the highest absolute metabolic rate in the human body, consuming approximately 20% of total body oxygen and generating the majority of its adenosine triphosphate through mitochondrial oxidative phosphorylation

within the electron transport chain complexes embedded in the inner mitochondrial membrane. Neurons, by virtue of their elaborate dendritic and axonal architecture, extensive synaptic signaling demands, and maintenance of ionic gradients through sodium–potassium adenosine triphosphatase activity, are critically dependent on a continuous and abundant supply of adenosine triphosphate; oligodendrocytes, which synthesize and maintain the myelin sheath, similarly require substantial metabolic energy for lipid biosynthesis and membrane turnover. Mitochondrial DNA encodes thirteen essential subunits of respiratory chain complexes I, III, IV, and V, and pathogenic mutations in these genes, whether point mutations in structural subunits such as MT-ND3, MT-ND5, and MT-ATP6, or transfer ribonucleic acid mutations such as MT-TL1 m.3243A>G that impair mitochondrial translation globally, result in diminished electron transport chain flux, reduced adenosine triphosphate synthesis, increased generation of reactive oxygen species, and impaired calcium buffering capacity. The resultant bioenergetic failure preferentially affects metabolically demanding neural structures: the basal ganglia and brainstem nuclei, which exhibit the highest mitochondrial density and oxidative metabolic rates in the brain, are characteristically and earliest affected in Leigh syndrome, explaining the symmetrical bilateral necrotic lesions observed on neuroimaging; the cerebral cortex and its vascular watershed zones are vulnerable in mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes, where the combination of energy failure, nitric oxide–mediated vasodilation, and blood–brain barrier disruption produces the characteristic stroke-like episodes. The tissue-specific threshold effect of heteroplasmy, whereby clinical manifestations emerge only when the proportion of mutant mitochondrial DNA molecules exceeds a critical threshold that varies by cell type and tissue, explains the highly variable clinical expressivity observed across the included studies, as different tissues achieve different mutation loads through stochastic mitochondrial DNA segregation during development. This mechanistic framework integrates the clinical observations of the present review: the preponderance of basal ganglia and brainstem involvement in Leigh syndrome cohorts, the association between higher heteroplasmy and worse prognosis reported by Danhelovska and colleagues, the correlation between basal ganglia magnetic resonance imaging involvement and mitochondrial DNA–specific diagnosis reported by Tolomeo and colleagues, and the limited involvement of mitochondrial DNA point mutations in autism spectrum disorder, a neurodevelopmental condition in which subtle synaptic and circuit-level dysfunction, rather than gross bioenergetic failure, is the predominant pathophysiological substrate.

The translational implications of this systematic review are relevant to several domains of pediatric clinical practice. First, the consistent identification of a relatively circumscribed set of recurrent mitochondrial DNA mutations across geographically and ethnically diverse populations, particularly MT-ATP6 m.8993T>G, MT-TL1 m.3243A>G, and complex I subunit mutations, supports the utility of incorporating mitochondrial DNA analysis into first-line molecular diagnostic algorithms for children presenting with Leigh syndrome, mitochondrial encephalomyopathy with stroke-like episodes, or unexplained progressive encephalopathy with lactic acidosis. Second, the markedly low diagnostic yield of targeted mitochondrial DNA mutation panels observed in the Egyptian cohort, contrasted with the substantially higher yield of comprehensive genome sequencing in the Australian cohort, provides a practical argument for the adoption of whole-genome or whole-exome-plus-mitochondrial-genome sequencing as the preferred diagnostic approach, particularly in populations with high consanguinity rates where nuclear DNA contributions may predominate. Third, the identification by Uusimaa and colleagues of a minimum carrier prevalence of 18.4 per 100,000 children for m.3243A>G in a Finnish population, with relatively low childhood morbidity but important

implications for adult-onset disease, underscores the need for long-term developmental surveillance of pediatric carriers identified through family screening or incidental genomic findings. Fourth, the conflicting evidence regarding mitochondrial DNA variation in autism spectrum disorder suggests that routine mitochondrial DNA testing is not warranted as a screening tool for all children with autism, but targeted evaluation may be appropriate for the subgroup of autistic children presenting with clinical features suggestive of mitochondrial dysfunction, including regression, epilepsy, exercise intolerance, or elevated lactate. Future clinical studies should employ standardized phenotyping instruments, comprehensive sequencing platforms, and multicenter collaborative designs to overcome the limitations of the current fragmented evidence base.

Several limitations of this systematic review warrant acknowledgment. First, the substantial heterogeneity across included studies with respect to study designs, populations, molecular testing methods, and outcome definitions precluded quantitative meta-analytic pooling, limiting the ability to generate summary effect estimates or evaluate publication bias statistically. Second, the majority of studies employed retrospective designs with recruitment from tertiary referral centers, introducing selection bias toward more severe or diagnostically complex presentations and limiting the generalizability of findings to the broader pediatric population. Third, molecular testing methods varied widely, from targeted polymerase chain reaction panels capable of detecting only a narrow set of known mutations to comprehensive whole-genome sequencing, and this methodological heterogeneity directly influences diagnostic yield estimates and mutation frequency comparisons across studies. Fourth, the absence of external comparator groups in many cohort studies limits the ability to assess the specificity of mitochondrial DNA findings relative to background population variation or non-mitochondrial disease controls. Fifth, several autism spectrum disorder studies included participants beyond the strict pediatric age range of 0–18 years, and pediatric-specific subgroup analyses were not always available, potentially introducing age-related confounding. Sixth, risk-of-bias assessment identified important concerns regarding allocation concealment, confounding adjustment, and incomplete outcome data across the majority of included studies. Despite these limitations, the consistency of phenotypic patterns and the convergent identification of key pathogenic mutations across diverse populations strengthen the overall conclusions of this review.

Conclusion

This systematic review demonstrates that mitochondrial DNA mutations are associated with a broad and clinically significant spectrum of pediatric neurological disease, with Leigh syndrome and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes representing the predominant phenotypic presentations across geographically and ethnically diverse populations. Recurrent mutations in MT-ATP6, MT-TL1, and mitochondrial DNA–encoded complex I subunit genes were consistently identified across independent cohorts, supporting their diagnostic and prognostic relevance. The evidence regarding mitochondrial DNA involvement in autism spectrum disorder remains inconclusive, with large genetic association studies failing to demonstrate a major population-level contribution despite smaller studies identifying mitochondrial DNA deletions and copy-number alterations in subsets of affected children. Methodological limitations including heterogeneous testing strategies, retrospective designs, and the absence of standardized phenotyping systems constrain the current evidence base. Future investigations should prioritize comprehensive mitochondrial DNA and nuclear genome sequencing in prospective, multicenter pediatric cohorts with standardized clinical phenotyping, long-term developmental follow-up, and tissue-specific heteroplasmy assessment to advance the understanding and clinical management.

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