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REVIEW

Systematic review of mitochondrial genetic variation in attention-deficit/hyperactivity disorder

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Abstract

The global prevalence of attention-deficit/hyperactivity disorder (ADHD) is estimated to be between 6% and 7% in children worldwide. The pathophysiology of this heterogeneous neurodevelopmental disorder remains unknown. Mitochondrial dysfunction has been proposed as a possible contributing factor to the etiology of ADHD. There is limited literature available to help our understanding of this hypothesis, and thus we conducted a systematic review of the number and quality of studies pertaining to mitochondrial genetic alterations in ADHD. A systematic search was conducted in the relevant databases Medline (PubMed) and Embase up to March 2021. Inclusion criteria included randomized control trials, cross-sectional studies, and case–control studies. This search resulted in a total of 507 articles that emerged from the search criteria. Of these results, 10 primary research articles were selected for in depth review based on the inclusion and exclusion criteria. These studies all reported on mitochondrial genetic variation in ADHD cases such as increased copy number, single-nucleotide polymorphisms, and haplogroup associations. This initial review of the experimental literature suggests mitochondrial genetic variation, in both the mitochondrial DNA and nuclear-encoded mitochondrial genes, may indeed contribute to ADHD pathophysiology. The studies reviewed here provide promising evidence for future research to further examine the mitochondrial genetics contributing to ADHD pathophysiology. We suggest that expansion of investigations into mitochondrial mechanisms may have potential to inform new treatment options for ADHD.

Keywords ADHD · Mitochondria · Mitochondrial genetics · Mitochondrial dysfunction · Neurodevelopment

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder that is characterized by a repeated pattern of inattention and/or hyperactivity–impulsivity that significantly impacts an individual's daily functioning and developmental trajectory [1]. The diagnosis of ADHD may specify whether an individual has either a combined, predominantly inattentive, or predominantly hyperactive/impulsive presentation [1].

Currently, the global prevalence of ADHD, which appears to be increasing, is estimated to be between 6% and 7% in children, and 2.5% in adults [2, 3]. It is estimated that 30–50% of cases persist into adulthood [4], demonstrating the significant burden of this neurodevelopmental disorder throughout the lifespan, while studies including occurrence of adverse life events have suggested possible gene x environment interactions in adult ADHD [5].

Twin and family studies have determined the heritability of ADHD is relatively high, at approximately 70–80% [6, 7]. Given the increasing prevalence and substantial heritability of ADHD, a review of the underlying genetic variants contributing to this disorder is warranted.

Identification of common genetic variants contributing to ADHD has previously been challenging. Originally, these variants were individually thought to contribute a very small amount to the overall heritability of ADHD [8]. One recent

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genome-wide association study (GWAS) of ADHD subjects versus controls used a large sample size with reasonable power, and identified 12 independent loci that passed genome-wide significance [9]. Moreover, an abundance of candidate gene studies have identified a variety of genes significantly associated with ADHD [10–12]. Although a significant proportion of the candidate genes identified have been serotonergic and dopaminergic genes [11–15], as well as neurogenesis, synaptogenesis, and synaptic activity related genes [16], a recent surge of evidence has identified mitochondrial genes as a potential target for ADHD [17]. These mitochondrial genes may represent a relevant genetic pathway that is altered in individuals with ADHD.

Mitochondria are organelles responsible for the production of energy (Adenosine triphosphate) in most human cells. Of relevance to neurodevelopmental disorders, such as ADHD, mitochondria regulate neuronal functioning, including neurotransmitter release, synaptic plasticity, and neuronal growth [18–20]. Thus, nocuous mitochondrial genetic variants may contribute to a multitude of deficits within the brain, which in turn may contribute to the development of ADHD.

The genes which encode mitochondrial proteins are found in both the nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) [21]. The majority of genes required for mitochondrial organelle assembly and function are found in the nDNA, whereas the double-stranded circular mtDNA encodes 37 genes, 13 of which are protein coding electron transport chain (ETC) genes [22, 23]. Of note, mtDNA is solely inherited from the maternal germline [21]. Additionally, not all mtDNA copies are identical and this mixed pool of mtDNA is defined as heteroplasmy [24]. High levels of heteroplasmy within the cell may be pathogenic [25]. Moreover, mtDNA can be analyzed based on its functional haplogroups [26], i.e., a group of individuals who have the same geographical maternal ancestry and share a specific set of mtDNA variants that were accumulated over time due to human migration worldwide[27]. Based on the above, it is not surprising that recent evidence has demonstrated mitochondrial genetic variation may contribute to ADHD pathophysiology.

ADHD clinical heterogeneity poses a challenge when attempting to categorize individuals into one diagnostic category. To address this issue, researchers have been searching for more clinical homogeneous subgroups of patients. Mitochondrial dysfunction may be a potential marker for a subtype of ADHD, given that multiple studies have shown children with the disorder display mitochondrial disturbances. For example, cell lines from ADHD individuals show significantly decreased mitochondrial respiration and oxidative phosphorylation (OXPHOS) activity, resulting in decreased ATP production in conjunction with impaired neurite outgrowth and branching [28–30]. Genetic studies are limited and to the best of our knowledge, there is no review examining published studies regarding mitochondrial genetic findings in ADHD. This review aims to close this gap by utilizing a systematic methodological approach to summarize the accumulated literature of mitochondrial genetics in ADHD, and provide future directions required to advance knowledge in the field.

The findings from this review will be useful for clinicians to follow the results and implications of studies that feature molecular and genetic factors reported as important in the etiology, triggering, or perpetuation of ADHD. Identification of genetic factors often leads to understanding of mechanisms through the known biological function of the specific genetic variation. These genetic factors might also serve as biomarkers for tracking severity and treatment effectiveness. Furthermore, the biological knowledge gained from the genetic variation and its function could lead to development of new drugs and alternative treatments targeted to mitochondrial processes, such as management of reactive oxygen species and overactive inflammatory responses.

Methods

A systematic literature search was conducted in accordance with the guidelines presented by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). First, the databases Medline (PubMed), and Embase were searched up to March 2021. Search strategy included using multiple terms for individual concepts, such as mitochondria, ADHD, and OXPHOS and then combining these with AND in OVID searches. Records were also identified through other sources, specifically ResearchGate and Google Scholar, which found eight additional articles that were not detected in the initial database searches. Table 1 outlines the specific search terms used in each database search, with appropriate text words, proximity operators, truncations, and search field codes.

After these searches were conducted, duplicate records were removed, and the subsequent articles were screened. The specified inclusion criteria were as follows; randomized control trials, cohort studies, case–control studies, must be published in a peer-reviewed journal, must be written in English, ages of sample used must be in the range of childhood to adulthood, and must include mitochondrial genetic examinations, in either the nDNA or mtDNA, with ADHD. Exclusion criteria included: case reports, opinion pieces, editorials, review articles and meta-analyses. Moreover, studies were excluded if they did not analyze genes directly related to mitochondrial functioning. Assessment of study quality, which was conducted on full-text articles being assessed for eligibility that passed the screening phase, included a review of the quality of the peer-reviewed journal,

sample size, types of controls used, and whether biases were present.

Results

Literature search

The literature search yielded 507 articles matching the search terms used, of which 10 articles passed all screening and eligibility criteria and were included (Figure 1). Of the 10 articles included, the majority of studies (N=6) examined mtDNA genes and polymorphisms, while the remaining studies analyzed nDNA mitochondrial genes and gene-sets to determine whether they were associated with ADHD.

Mitochondrial DNA (mtDNA) findings in ADHD

The first piece of evidence recognizing mtDNA polymorphisms may be associated with ADHD was published only recently, by Hwang et al. 2017, which analyzed a sample of Korean childhood ADHD cases versus controls [31]. Given the reported association of mtDNA 10398 A/G polymorphism with bipolar disorder [32], Alzheimer's disease [33], and Parkinson's disease [34], Hwang et al., decided to exclusively examine this polymorphism to determine whether a similar association exists with ADHD. This study analyzed 120 ADHD cases diagnosed by child psychiatrists using the DSM-IV with a mean age of 8.05, and 322 age-matched controls. Phenotypes of adaptive and problem behaviors were assessed using the Behavior Assessment System for Children-Second Edition (BASC-2) rating system, where only the Parent Rating Scale (PRS) was used. Hwang et al., showed that indeed, mtDNA 10398 A/G polymorphism was associated with ADHD (OR 0.63, $p = 0.024$), as well as with aggression behavior and leadership in boys with ADHD ($p = 0.040$ and $p = 0.037$, respectively). Interestingly, no association was observed when analyses were stratified for gender and ADHD subtype. Given their preliminary findings, Hwang et al. 2019, conducted a secondary haplogroup analysis in their sample [35]. Results showed that haplogroup B4 and B5 ($p = 0.031$, OR 1.90 and $p = 0.041$, OR 0.26, respectively) were significantly associated with an increased risk of ADHD. In addition, ADHD subtype analysis revealed haplogroup B4 was strongly associated with the combined subtype ($p = 0.007$, OR 2.50), and ADHD diagnosis in females is associated with the D4b haplogroup ($p = 0.014$, OR 4.83) compared to control females. Furthermore, the sample size used by Hwang et al. 2019 was calculated to obtain over 95% power based on previous literature outlining

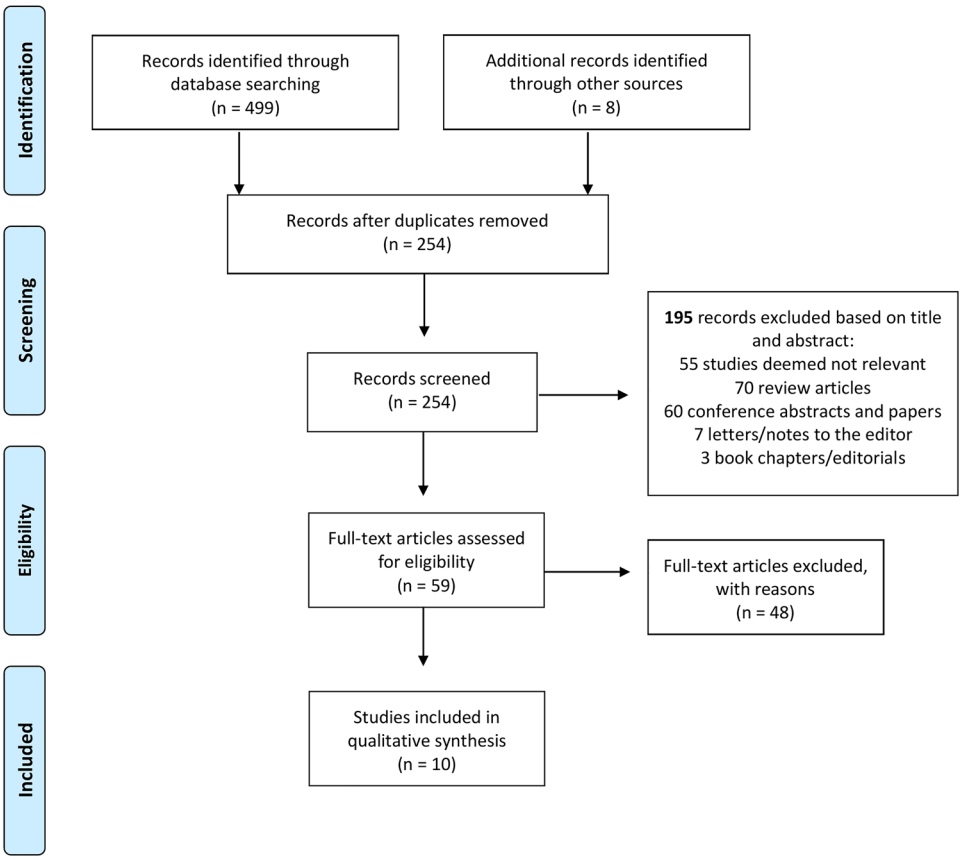

Fig 1. PRISMA guided literature search flow diagram outlining the steps taken to select studies included in systematic review

sample size requirements for ADHD cases and controls in the Korean population [36].

Another mtDNA finding was published by Kim et al. 2019, where they examined 70 Korean ADHD cases, with an average age of 9.8 +/- 2.6, versus 70 age-matched healthy controls [37]. ADHD diagnosis was confirmed by child psychiatrists using the Korean Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime version (K-SADS-PL) [38]. Additionally, the parent-report Korean version of the ADHD Rating Scale-IV (ADHD-RS) was used in this study to assess ADHD severity. Results showed that mtDNA copy number, which was measured based on the ratio of mtDNA to nDNA, was nominally significantly increased in ADHD cases ($p = 0.028$) compared with healthy controls. Moreover, methylation of the peroxisome-proliferator-activated receptor gamma co-activator -1 alpha (*PPARGC1A*), a co-transcriptional regulation factor involved in mitochondrial biogenesis, and the D-loop region of mtDNA, a non-coding region that works as a promoter site and is a main site of mtDNA variation, were measured. ADHD cases only displayed a significantly decreased methylation ratio of the *PPARGC1A* promoter region ($p = 0.008$), but not in the D-loop region. Finally, no mtDNA copy number differences or methylation ratios were observed among ADHD subtypes and scores on ADHD-RS.

mtDNA alterations associated with ADHD do not seem to be solely attributable to Korean individuals, in fact recent reports have replicated these findings in Caucasian samples. Ögütlü et al. 2020, similarly showed that average mtDNA copy number, measured by the ratio of mtDNA to nDNA, was significantly increased in ADHD cases ($n = 56$, $p = 0.002$) versus age- and sex- matched controls ($n = 56$)[39]. Ögütlü et al. 2021, conducted a one-year follow-up study on their initial sample to determine the effects of treatments and ADHD severity on mtDNA copy number [40]. 28 ADHD patients who participated in the first study were included, and cases were divided into treated ($n = 14$) and non-treated ($n = 14$) groups. This follow-up study showed the average mtDNA copy number did not change significantly over one year, or between the two groups. The Conners' parent rating scale (CPRS) was used to measure ADHD severity, and mtDNA copy number was significantly correlated ($p = 0.049$) with CPRS ADHD index and inattention scores, but only in the treated group.

Finally, Chang et al. 2020, recently examined whether mtDNA haplogroups influenced the risk of ADHD in a large sample of individuals of Caucasian descent [41]. Here, a total of 2076 ADHD cases, diagnosed by the K-SADS interview, and 5078 age- and sex-matched healthy controls were collected from three separate ADHD cohorts. HaploGrep 2 was used to assign haplogroups, and only common European haplogroups were selected for analysis, including: H,HV, I, J, K, OX, T, U, and W. This study found that haplogroup K in the first cohort ($n = 764$ cases and 2031 controls) was significantly decreased (OR 0.64, $P_{corrected} = 0.035$) in ADHD cases, possibly indicating a protective role. Furthermore, haplogroup UK, which was combined as a super-haplogroup given that haplogroup K was derived from haplogroup U was found to be significantly associated (OR 0.66, $P_{corrected} = 2.46 \times 10^{-3}$) with ADHD risk in cohort 2 ($n = 651$ cases and 2003 controls). Lastly, the haplogroup UK association with ADHD risk was replicated in the last cohort ($n = 661$ cases and 1044 controls, OR 0.7, $P_{corrected} = 5.85 \times 10^{-3}$), and a meta-analysis combining all three cohorts revealed that haplogroups UK was significantly associated with ADHD risk after multiple testing correction. The meta-analysis also showed that haplogroup HHV was significantly associated with an increased risk of ADHD (OR 1.176, $P_{corrected} = 0.0185$). Taken together, the recent evidence summarized here suggests mtDNA alterations may contribute to ADHD risk (Table 2).

Nuclear mitochondrial genetic variation in ADHD

Given the mtDNA encodes only 37 mitochondrial genes, most genes required for mitochondrial assembly and functioning are encoded by the nDNA. The nDNA encodes ~1000 mitochondrial genes [42], of which both genes and gene-sets have been associated with ADHD. Initial investigation by Lesch et al. 2011, aimed to examine whether micro-deletions and micro-duplications could be identified in 99 children with ADHD [43]. To conduct this study, a genome-wide screen was used to detect copy number variants (CNVs) (large genomic structural variations that can include deletions, duplications, and translocations [44]), in genes potentially involved in the psychopathology of ADHD. Interestingly, mitochondrial gene NADH dehydrogenase 1 alpha subcomplex assembly factor 2 (*NDUFAF2)* , and uncoupling protein 2 (*UCP2)* were identified as a candidate genes with CNVs associated with ADHD. Specifically, *NDUFAF2* was classified as aberrant due to inheritance of deletion 5q12.1 from an affected mother, in one male patient.

One genome-wide association study (GWAS) aimed to identify key genetic pathways that may contribute to the ADHD phenotype [45]. To identify both single-nucleotide polymorphisms (SNPs) as well as candidate gene pathways involved in ADHD, Lee & Song, 2014 used a sample of 924 European ancestry ADHD–parent–sibling trios . The improved gene-set enrichment analysis (*i*-GSEA), was used to conduct an annotation of biological mechanisms to preselected candidate causal SNPs, identified by linkage disequilibrium (LD) analysis, which was then used to conduct ICSNPathway analysis [46]. The SNPs and genes identified by the GWAS pathway analysis included the mitochondrial translation release factor in rescue (*C12orf65*) gene, which encodes a mitochondrial matrix protein contributing to

Table 2. Main findings in mtDNA ADHD systematic review search

Study Design	Sample Size	Ethnicity	Main Findings	Reference
ADHD cases versus healthy controls	120 ADHD cases, 322 age-matched healthy controls	Korean	Mitochondrial DNA 10398 A/G polymorphism was significantly associated with ADHD. 10398 A/G polymorphism was associated with aggression behavior and leadership on BASC-2 scale in ADHD boys	Hwang et al. (2017)
ADHD cases versus healthy controls	70 ADHD cases, 70 healthy controls	Korean	mtDNA copy number significantly higher in ADHD cases. ADHD subtypes had a significant difference in methylation ratio of *PPARGC1A* promoter region, with the combined subtype displaying the highest methylation ratio	Kim et al. (2019)
ADHD cases versus healthy controls	120 ADHD cases, 322 healthy age-matched healthy controls	Korean	Haplogroup B4 was significantly associated with ADHD. Male ADHD cases were associated with haplogroup B5. Lastly, combined ADHD subtype was associated with haplogroup B4	Hwang et al. (2019)
ADHD cases versus healthy controls	56 ADHD cases, 56 age-matched healthy controls	Turkish	Mean mtDNA copy number was significantly increased in ADHD cases	Ögütlü et al. (2020)
ADHD case severity; 1 year follow-up	28 ADHD patients	Turkish	mtDNA copy number remained similar in all ADHD patients after one year. Significant correlation between CPRS ADHD index, inattention scores, and mtDNA copy number in medication treated group	Ögütlü et al. (2021)
ADHD cases versus healthy controls	2076 ADHD cases, 5078 age- and sex-matched healthy controls	European	European mitochondrial haplogroups K and U were significantly associated with increased risk of ADHD	Chang et al. (2020)

Acronyms: Attention-Deficit/Hyperactivity Disorder (ADHD), mitochondrial DNA (mtDNA), Peroxisome Proliferator-Activated Gamma Coactivator 1-Alpha (*PPARGC1A*), Behavior Assessment System for Children-Second Edition (BASC-2), Conners' Parent Rating Scale (CPRS)

mitochondrial translational machinery. Given this finding, along with calculations to determine the biological pathway associated with this gene, the mitochondrial pathway, specifically the role of mitochondria in apoptotic signaling, was the second strongest pathway associated with ADHD (nominal p <0.001, FDR = 0.032).

Animal model studies have been used to better understand the underlying genetic alterations that may occur in the brain, which in turn may lead to the development of ADHD. One such study aimed to identify the consequences of long-term methylphenidate treatment used for ADHD [47]. To assess this, a validated rat model of ADHD (spontaneously hypertensive rats), were treated with methylphenidate during adolescence and subsequently gene expression in the prefrontal cortex (PFC) and striatum was analyzed using genome-wide transcriptome profiling analyses. Using the Affymetrix GeneChip microarray, RNA samples were converted to complimentary DNA (cDNA) libraries, allowing for gene expression to be analyzed. Gene expression in the striatum showed differential effects regarding mitochondrial functionality, where mtDNA NADH dehydrogenase subunit 2 (*ND2*) gene was upregulated. Conversely, a plethora of nuclear mitochondrial organization genes (n = 12) were downregulated in the striatum, including the ubiquinol cytochrome c reductase core protein 2 (*Uqcrc2*) gene, which encodes a component of OXPHOS complex III.

Animal models are not the only study design that have been used to understand ADHD, in fact, Palladino et al. 2020 used patient-derived fibroblasts to study the effect of rare CNVs in the parkin RBR E3 ubiquitin protein ligase (*PARK2*) locus on ADHD cellular function [28]. The *PARK2* gene is necessary for the correct functioning of mitochondrial quality control systems such as mitophagy (the selective degradation of dysfunctional mitochondria), mitochondrial transport, maintaining redox balances within the cell, and importantly mitigating the mitochondrial oxidative stress response [48]. The sample consisted of four ADHD cases (three with known CNVs, one ADHD case without, used as a wild-type ADHD control) and two healthy controls. These individuals provided skin punch biopsies, used for the collection of fibroblasts, which were then reprogrammed into human-induced pluripotent stem cells (hiPSCs). These hiPSCs were further differentiated into dopaminergic neuronal cells (mDANs), which could be used to assess in-vitro neuronal functioning of ADHD individuals with rare *PARK2* CNVs. Interestingly, when cell cultures were exposed to a starvation stress paradigm, aimed to target mitochondrial functioning and energy metabolism, both *PARK2* deletion and duplication carrier fibroblast cell lines showed decreased levels of *PARK2* protein compared to healthy controls (Tukey HSD for both comparisons $p \leq 0001$). Moreover, in the mDAN cell lines the healthy controls displayed an increase in *PARK2* protein levels compared to the *PARK2* deletion and duplication carriers ($p = 0.024$) at baseline. Therefore, there is evidence indicating CNVs affecting the *PARK2* locus may contribute to mitochondrial dysfunction underlying the pathophysiological mechanisms contributing to ADHD. Table 3 outlines the main mitochondrial nDNA findings reported here.

Table 3. Main findings in mitochondrial nuclear DNA ADHD systematic review search

Study Design	Sample Size	Main Findings	Reference
Genome-wide copy number analysis in ADHD cases	99 children with ADHD	Mitochondrial gene *NDUFAF2,* and *UCP2* deletion was observed in ADHD cases	Lesch et al. (2011)
Genome-wide pathway analysis in ADHD–parent–child trios	924 ADHD-parent-sibling trios, 2,758 total individuals	Mitochondrial apoptotic signaling gene pathway identified as second strongest mechanism in ADHD cases	Lee & Song (2014)
ADHD rat model versus healthy control	21–27 rats, divided into controls, and ADHD SHR rats	Mitochondrion organization genes were downregulated in the striatum of ADHD rat models treated with methylphenidate	Peña et al. (2014)
ADHD patient-derived fibroblast, reprogrammed to pluripotent stem cells and differentiated into dopaminergic neuronal cells versus healthy control cell lines	4 ADHD cases, 2 healthy controls	*PARK2* CNV deletion and duplication carriers with ADHD showed nominally lower *PARK2* gene expression	Palladino et al. (2020)

Acronyms: Attention-Deficit/Hyperactivity Disorder (ADHD), NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 2 (*NDUFAF2*), Uncoupling Protein 2 (*UCP2*), Spontaneously hypertensive rats (SHR), Parkin RBR E3 Ubiquitin Protein Ligase (*PARK2*)

Discussion

The role of mtDNA genetic variants in ADHD pathophysiology

Given the significant heritability rate of ADHD, it is not surprising genetic variants have been implicated in ADHD pathophysiology. Recent advances in high throughput sequencing have allowed for more sophisticated characterization of the mitochondrial genome, resulting in intriguing findings [26]. The recent studies published and reviewed here regarding mtDNA variation associated with ADHD illustrate genetic variants may be particularly valuable when attempting to understand how ADHD arises during development. One of the findings, which was replicated independently in two separate samples, was an increase of mtDNA copy number in ADHD cases versus controls. Emerging research has shown that mtDNA copy number may serve as a biomarker for toxic effects of stress in the body [49]. Increased stress can lead to downstream cascade effects on a variety of cellular processes. Mitochondrial function is one of the main pathways that may be affected, with an increase in reactive oxygen species (ROS) [50]. Specifically, stress causes glucocorticoid secretion, which if continually activated can lead to pathological inflammation at the molecular level, resulting in increased ROS [51]. An accumulation of oxidative stress has been proposed as a potential mechanism contributing to a variety of psychiatric disorders, due to the damaging effects redox imbalance can pose to the brain [52]. Given the increase in mtDNA copy number observed in ADHD cases, oxidative stress may similarly be contributing to the underlying pathophysiology of ADHD, especially considering there is an increased susceptibility of the brain to oxidative imbalances during the developmental years. Important to note, increased mtDNA copy number was observed in only two of the six mtDNA studies reviewed here, and given the modest sample size of these studies, replication in a larger cohort of ADHD patients versus healthy controls is required.

The next key finding observed in two of the six mtDNA studies was haplogroup associations with ADHD. Interestingly, both Asian haplogroups B4 and B5, (Hwang et al., 2019) and European haplogroup U (Chang et al., 2020) were significantly associated with ADHD risk. European haplogroups have previously been associated with neurodevelopmental disorders such as Autism Spectrum Disorder [53], and Asian haplogroups have been associated with Alzheimer's disease [27]. Haplogroups may represent a unique dimension of influence on neurodevelopmental disorders and neurodegenerative diseases. Given that two independent studies identified distinct ancestral haplogroups to be associated with ADHD, mtDNA lineages, and their respective variants, may represent a novel susceptibility factor contributing to ADHD risk.

Only one mtDNA polymorphism was identified by our literature search, which was the mtDNA 10398 A/G site. This polymorphism is within the mitochondrially encoded *ND3* gene, which encodes NADH dehydrogenase subunit 3, a component of complex I of the OXPHOS system. Complex I is the location of free radical generation, whereby increased superoxide production in both rat and human brain mitochondria is observed [54]. Furthermore, in animal models decreased free radical production is observed during inhibition of complex I with rotenone [55]. Variation in these genes may contribute to variations in ROS production, which at low to moderate levels could be advantageous to the long-term adaptability of a species [50]. Alternatively, more extreme perturbations of ROS may lead to pathological effects that, over childhood development, are manifested by behavioral dysregulation and ADHD [56]. In view of the current literature, mtDNA genetic variability, including mtDNA copy number, haplogroup variability, and SNPs, may contribute to ADHD risk, although more studies are still warranted.

Nuclear-encoded mitochondrial genetic variations and the heritability of ADHD

Nuclear-encoded mitochondrial genes make up the vast majority involved in mitochondrial functioning. However, it should be noted the mitochondrial genome exists in many more copy numbers (10–1000) than the single nuclear genome present in a typical cell. These genes all work in tandem to ensure that normal mitochondrial and cellular functioning is carried out within the cell. Publications focusing on these nuclear-encoded mitochondrial genes often specify a certain set of genes relevant to a given mitochondrial pathway, for example the mitochondrial-apoptotic pathway. Lee & Song (2014) indeed identified the mitochondrial-apoptotic signaling gene pathway as one of the strongest associations to ADHD in a parent–sibling–proband sample. The mitochondrial-apoptotic signaling pathway is, in all likelihood, relevant when considering brain functioning, neuronal growth, and synaptic activity [57]. Because the mitochondria regulate Ca^{2+} homeostasis and signaling, they also in turn regulate cell death (apoptosis) through the accumulation of Ca^{2+} in mitochondria and release of cytochrome c, triggering the apoptotic cascade [58]. Moreover, the regulation of Ca^{2+} by mitochondria is critical for neurogenesis, neuronal plasticity, synaptic homeostasis, and regulation of neurotransmission [59]. Dysregulation of apoptosis has been associated with numerous neurodegenerative diseases [59]. This apoptotic dysfunction may negatively affect certain brain regions required for sustained attention and proper

regulation of activity levels. Thus, apoptotic dysregulation, underlined by variation in nuclear-encoded mitochondrial-apoptotic genes, may be contributing to the heterogeneous presentations of ADHD.

Key findings in the other nuclear genetic studies reviewed here include variation in *NDUFAF2* and *UCP2*, downregulation of the mitochondrial organization network and the *PARK2* gene. Therefore, variation in mitochondrial function and organization genes may lead to disturbed mitochondria, resulting in changes in OXPHOS and increased oxidative stress within the ADHD brain. The studies reviewed regarding nuclear mitochondrial findings in ADHD, ranged from large sample GWASs, to hiPSC's and rat models. Although using a variety of study designs is beneficial when attempting to dissect the underpinnings of a complex neurodevelopmental disorder such as ADHD, it poses a challenge for direct comparison of the results. Additionally, the limited number of publications reporting nuclear-encoded mitochondrial genetic abnormalities in ADHD, as well as the relatively small sample sizes used, pose further challenges for interpretation of the results. Importantly, it is necessary to consider the role of publication bias in a systematic review. Although efforts were made to search all relevant databases and identify both positive and negative results in the literature, there often can be a bias towards publication of positive results, which may be why all studies reviewed here report positive associations of one type or another with ADHD.

Outlook and future directions

This systematic review has outlined a variety of studies that report mitochondrial genetic variants are indeed associated with ADHD risk. Notably, most GWAS do not include the mtDNA in their analyses due to analytical complexities produced by the unique features of this genome. The mitochondrial genome is maternally inherited. There are many copies of mtDNA inside a given cell and not all copies are identical, resulting in varying levels of heteroplasmy [24]. Furthermore, the mitochondrial genome is not diploid like the nuclear genome, and thus most of the standard genetic analysis software is not designed to address the haploid mitochondrial genome. In addition, the machinery used for the replication of mtDNA, which most likely includes multiple concurrent models [60], seems to be slightly inefficient compared to the nuclear genome given the reduction in mtDNA replication speed observed [61]. For all these reasons, standardized analytical procedures on how to approach analysis of the mtDNA genome are required.

In this review, we described key research findings regarding the potential role of mitochondrial gene variants and haplogroups in ADHD risk and clinical severity. Future research guided by these findings should consider searching for causal SNPs inside of significant associated haplogroups, particularly haplogroup UK. This approach may involve next-generation sequencing to detect rare variants or potential heteroplasmic sites in LD with haplogroup-defining SNPs. The search through multiple ancestral backgrounds would shed light on the penetrance of the candidate variants and their applicability as universal markers for ADHD. Furthermore, the use of a hypothesis-driven approach to examine nuclear-encoded mitochondrial gene pathways in ADHD may be valuable given the evidence presented here regarding nuclear-encoded mitochondrial genes. Although these findings are promising, an examination of whether these results can be replicated in larger samples is required. These approaches have the potential to inform new treatment options for ADHD.

Appendix

See Appendix Table 1

Table 1. Search terms used in Embase and Medline database searches

Embase Searches	Medline Searches
1. (oxidative phosphorylation or OXPHOS).mp.	1. mitochondri*.tw,kf.
2. (mitochondri* adj2 (geneti* or gene or DNA)).mp.	2. (oxidative phosphorylation or OXPHOS).tw,kf.
3. mitochondr*.mp.	3. (mitochondri* adj2 (geneti* or gene or DNA)).tw,kf.
4. (mitochondr* adj2 (dysfunc* or disturb* or abnorm*)).mp.	4. exp Attention Deficit Hyperactivity Disorder/
5. Attention Deficit Hyperactivity Disorder/	5. Attention Deficit Hyperactivity Disorder/
6. ADHD.mp.	6. ADHD.mp.
7. attention defi* hyperacti* disor*.mp.	7. mitochondri*.mp.
8. 1 OR 2 OR 3 OR 4	8. attention defi* hyperacti* disor*.mp.
9. 5 OR 6 OR 7	9. (mitochondr* adj2 (dysfunc* or disturb* or abnorm*)).mp.
10. 8 AND 9	10. 1 OR 2 OR 3 OR 7 OR 9
	11. 4 OR 5 OR 6 OR 8
	12. 10 AND 11

Author contributions **SVG** conceived and designed the review, conducted the systematic search, reviewed all articles produced by the review and determined eligible studies, and contributed with text review and editing. **DM** conducted a critical review of the manuscript, **JLK** conducted a critical review of the manuscript and contributed with text review and editing. **VFG** was involved with supervision of the review and conducting a critical review of the manuscript along with edits.

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Availability of data and materials All studies reviewed here are available on Embase and Medline.

Code availability Not applicable.

Declarations

Conflict of interest JLK and VFG are co-authors on a patent that includes mitochondrial markers, for prediction of antipsychotic side effects.

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