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Epigenetics of methylmercury

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ABSTRACT

Purpose of review: Methylmercury (MeHg) is neurotoxic at high levels and particularly affects the developing brain. One proposed mechanism of MeHg neurotoxicity is alteration of the epigenetic programming. In this review, we summarise the experimental and epidemiological literature on MeHg-associated epigenetic changes.
Recent findings: Experimental and epidemiological studies have identified changes in DNA methylation following *in utero* exposure to MeHg, and some of the changes appear to be persistent. A few studies have evaluated associations between MeHg-related changes in DNA methylation and neurodevelopmental outcomes. Experimental studies reveal changes in histone modifications after MeHg exposure, but we lack epidemiological studies supporting such changes in humans. Experimental and epidemiological studies have identified microRNA-related changes associated with MeHg; however, more research is needed to conclude if these changes lead to persistent and toxic effects.
Summary: MeHg appears to interfere with epigenetic processes, potentially leading to persistent changes. However, observed associations of mercury with epigenetic changes are as of yet of unknown relevance to neurodevelopmental outcomes.

1. Introduction

Exposure of humans to methylmercury (MeHg) occurs mainly via consumption of fish and to a lesser extent via rice (Yang et al., 2021). MeHg is taken up easily in the gastrointestinal tract and readily passes the placenta and the blood-brain barrier (Nordberg and Costa, 2021). Exposure to high levels of MeHg early in life leads to irreversible neurotoxic effects (Bakir et al., 1973; Harada, 1995). However, results from epidemiologic research on associations between low-to-moderate levels of MeHg exposure from fish and children's neurodevelopment are inconsistent (Barbone et al., 2019; Grandjean et al., 1997; Gustin et al., 2017; Llop et al., 2012; Rothenberg et al., 2021; Strain et al., 2021; van Wijngaarden et al., 2017; Vejrup et al., 2016). Possible reasons for these mixed findings include diversity in study designs and populations studied, heterogeneity of fish and rice consumption patterns across populations, beneficial factors in fish, co-exposure to other toxicants, and differences in genetics. Epigenetics may well be another explanation for such differences.

Epigenetic marks regulate temporal and spatial patterns of transcription and play a critical role in cell differentiation and tissue organisation during general and neurological development (Bale, 2015; Cantone and Fisher, 2013; Eckersley-Maslin et al., 2018). Epigenetic marks are reversible and respond to different endogenous and exogenous signals, and epigenetics may therefore represent the dynamic missing link between genes and the environment. However, some epigenetic marks can be propagated from one generation of cells to the next and thus can exist as heritable memory in the cell. Epigenetic regulation has been suggested to affect vulnerability to brain disorders (Iraola-Guzmán et al., 2011).

DNA methylation, the addition of a methyl group to the 5′ position of cytosine (5-methylcytosine, 5mC) in the context of cytosine-guanine dinucleotides (CpGs), remains the most extensively studied epigenetic alteration in mammals. Global DNA methylation refers to the total level of 5mC relative to total cytosine in a sample, and may reflect gross changes in DNA methylation. Gene-specific DNA methylation focuses on specific chromosome regions or pathways.

Changes in post-translational modifications of histones and microRNA (miRNA)-mediated mechanisms are receiving increasing attention. Histones (numbered H1–5) are nuclear proteins around which DNA winds. Post-translational modifications of histones, including

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methylation and acetylation, affect how tightly the DNA is wrapped and in turn how accessible it is for transcription, recombination, and replication (Millán-Zambrano et al., 2022). MiRNAs are noncoding RNAs of ~22 nucleotides, which inhibit the translation of specific target messenger RNAs (mRNAs), or activate the translation of other mRNAs (Ni and Leng, 2016). MiRNAs play a role in embryonic development, signalling pathways, and apoptosis, among other cellular processes, and altered miRNA levels have been associated with cognitive disorders (Nadim et al., 2017). In plasma, miRNAs are protected from endogenous RNAse activity and are stable. Hence, they may serve as biomarkers of toxicity and disease.

Early-life exposure to metals such as lead, arsenic, and cadmium, is associated with differential DNA methylation patterns (Engström et al., 2015; Gliga et al., 2018; Kippler et al., 2013), and in vivo experiments have demonstrated that arsenic and cadmium interact with DNA methyltransferases (Bommarito et al., 2017; Comparative Toxicogenomics Database: www.ctdbase.org). Several recent experimental (reviewed by Culbreth and Aschner, 2019; Ke et al., 2023) and epidemiological studies indicate that MeHg also interacts with the epigenome. This review summarizes the current knowledge of experimental, followed by epidemiological, studies about MeHg exposure and its relation with each of the epigenetic marks: DNA methylation, histone modifications, and miRNAs.

2. Method

We compiled existing published knowledge relevant for this review up until Aug 2022. Before the searches begun, possible keywords (mercury/ methylmercury + epigenetic/ DNA methylation/ histone/ microRNA/ noncoding RNA) were defined (step 1) based on discussions between the researchers. The searches (step 2) were conducted through Google Scholar and PubMed. Relevant literature was first identified based on titles (step 3a), then by abstracts (step 3b), before articles were selected for full reading (step 3c). In addition, relevant references were selected from identified articles. A flowchart of the screening and selection of articles is found in Supplementary Figure 1. Statistical significance refers to $p < 0.05$ or when appropriate, after corrections for multiple testing.

3. MeHg and DNA methylation

3.1. Animal and in vitro studies

Experimental studies have described the effects of MeHg on global DNA methylation, measured as 5mC (Table 1). Go et al. (2021) evaluated the effect of exposure to low concentrations of MeHg on DNA methylation, DNA methyltransferase levels, and their correlation with neuronal development in vitro and in vivo. For this, human dopaminergic neurons (LUHMES cell-line), were exposed to 1 nM MeHg for 6 days leading to increased global DNA methylation and higher levels of the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B (Go et al., 2021). This was validated in vivo in the same study, where C57BL/6 J mice dams were dosed orally with MeHg (3 mg/kg/day) during gestation. Brain tissue from their offspring at embryonic day 19 showed a global increase in DNA methylation in the cerebral cortex concomitant with increased protein levels of DNMT1 (Go et al., 2021). Decreased neurite length, an indicator of impaired neuronal development, was observed under both in vitro and in vivo conditions, and was rescued by co-treatment with DNMT inhibitor, indicating a direct link between the observed epigenetic changes and cellular outcomes. These findings are supported by a study in which pregnant mice were exposed to MeHg (4, 8, or 12 mg/L every other day in drinking water) during gestation (from gestational day 0 to gestational day 18), and their offspring were evaluated at embryonic day 18 for gross anatomical and molecular effects. Although all tested concentrations produced teratogenic effects in the gross examination, loss of post-mitotic neurons and epigenetic modifications were only evidenced in the 8 and 12 mg/L groups. In addition, foetuses from the 8 mg/L exposure group showed elevated levels of DNA methyltransferases together with increased global DNA methylation in the brain (Pan et al., 2022). A complementary in vitro examination showed that exposure of mouse hippocampal neurons (HT22 cells) to 1, 2, or 4 μM MeHg for 24 h decreased axonal length and increased the protein levels of DNMT1 and DNMT3A only at 4 μM, but those of DNMT3B at all concentrations tested (Pan et al., 2022).

Conversely, reduced global DNA methylation has also been reported after MeHg exposure. Primary rat embryonic cortical neural stem cells exposed to 2.5 or 5 nM MeHg for 48 h showed reduced global DNA methylation and *Dnmt3b* mRNA levels, a phenomenon also observed in cells from subsequent passages that were not directly exposed to MeHg (Bose et al., 2012). A decrease in global DNA methylation accompanied by decreased DNMT activity was also reported in brain tissue of juvenile male mink exposed to 1 ppm MeHg for 3 months (Basu et al., 2013). However, whole brains from developmental exposure (from gestational day 0–19) of chicken embryos as well as chronic exposure (twice a day for 4 weeks) of female yellow perch did not result in changes in global DNA methylation or in DNMT activity or expression (Basu et al., 2013). In these studies, no attempts were made to strengthen in vitro with in vivo findings or vice versa, nor to reverse the effects as was done in several studies reporting global increase.

Desaulniers et al. (2009) reported no changes in global DNA methylation in livers of female rats developmentally exposed (from gestational day 1 to postnatal day 21) to 0.02 or 2 mg/kg/day MeHg, although a reduction in *Dnmt1* and *Dnmt3* expression was found in the high exposure group. However, when investigating specific loci, these authors found reduced DNA methylation of the *Cyclin-dependent kinase inhibitor 2 A* (*Cdkn2a*) promoter in livers of female offspring. Still, gene expression changes were not observed for one of *Cdkn2a* transcripts ($p16^{INK4a}$) evaluated in this study, and no correlation to physiological effects were made (Desaulniers et al., 2009). In another study of loci-specific changes of MeHg, in kidneys of rats, adult females were exposed to 0.5 or 5 ppm MeHg by oral gavage for 28 days. DNA methylation decreased at two CpG sites in the exon 1 promoter of *Matrix metalloproteinase-9* (*Mmp9*), encoding an enzyme with proteolytic functions (Khan et al., 2017). The rats also showed increased *Mmp9* gene and protein expression, together with other molecular indicators of renal damage. For instance, the 0.5 ppm exposure group showed increased serum creatinine and histological renal lesions together with increased reactive oxygen species (ROS) and increased expression of hypoxia and proinflammatory markers (Khan et al., 2017). These results were complemented by cellular studies (NRK-52E; epithelial rat kidney cells) in which co-exposure to 1 μM MeHg and 100 nM SB-3CT (a Mmp9 inhibitor) decreased the protein levels of Mmp9, but DNA methylation was not studied in the in vitro model (Khan et al., 2017). As Mmp9 is involved in renal dysfunction, this study suggests that MeHg-induced epigenetic changes may have functional consequences in the kidney (Wozniak et al., 2021).

DNA methylation changes at specific loci have also been observed in neurodevelopmental models. In an in vitro model of dopaminergic neurons (SH-SY5Y cells; human neuroblastoma cell-line), 6 days of exposure to 8 nM, but not 40 nM, MeHg increased DNA methylation at *Mediator of RNA polymerase II transcription subunit* (*MED31*), encoding a predicted transcriptional activator (Cediel-Ulloa et al., 2022). This gene was identified from an epigenetic genome-wide association study (EWAS) on newborns and children exposed prenatally to MeHg from fish (Lozano et al., 2022), where *MED31* showed higher and persistent methylation with more MeHg. This study used concentrations derived from human exposure and strengthened epidemiological observations, however DNA methylation alterations did not correlate with gene expression changes, and the sample size was limited.

In male C57BL/6/Bkl mice exposed to 0.5 mg/kg/day MeHg from gestational day 7 to day 7 after delivery, increased DNA methylation at promoter IV of *Brain-derived neurotrophic factor* (*Bdnf*) was found in the

Table 1
Experimental studies of DNA methylation, histone modification, and microRNA expression.

Reference	Species	Experimental model	MeHg exposure duration and concentration	Epigenetic findings
DNA methylation				
Onishchenko et al. (2008)	Mouse (*Mus musculus*)	C57BL/6/Bkl	Gestational (embryonic day 7 until postnatal day 7); 0.5 mg/kg/day	Increased DNA methylation of *Bdnf* promoter IV with decreased *Bdnf* expression. Increased H3K27me and decreased H3 acetylation at *Bdnf* promoter IV.
Desaulniers et al. (2009)	Rat (*Rattus norvegicus*)	Sprague-Dawley, female	Developmental (from gestational day 1 to postnatal day 21); 0.02 or 2 mg/kg/day	Decreased *DNMT1* and *DNMT3B* expression. Decreased DNA methylation in the promoter of *Cdkn2a*.
Bose et al. (2012)	Rat	Primary embryonic cortical neural stem cells (NSCs)	Chronic (48 h); Parent NSCs (P1) exposed to 2.5 or 5.0 nM	Decreased global DNA methylation and decreased *Dnmt3b* expression.
Basu et al. (2013)	Mink (*Neovison vison*), chicken (*Gallus gallus*), and yellow perch (*Perca flavescens*)		Mink: Chronic (3 months); 0.1, 0.5, 1, and 2 mg/kg/day Chicken: Gestational (from embryonic day 0–19); 0.62, 2.0, 3.2, and 6.4 μg/g/day Yellow perch: Chronic (4 weeks); 0.5, 5, and 50 μg/g twice a day	Mink: decreased global DNA methylation and DNMT activity. Chicken and yellow perch: no statistically significant effects.
Olsvik et al. (2014)	Zebrafish (*Danio rerio*)		Transgenerational (F0, F1 and F2), females (F0) were exposed for 47 days to 10 mg/kg/day	No changes in global DNA methylation. In F0, 49 genes were hypomethylated. In F1, 32 genes were differentially methylated, and in F2, 1 gene was hypermethylated.
Khan et al. (2017)	Rat	Wistar, adult female	Chronic (28 days); 0.5 or 5 ppm	Hypomethylation in the promoter of *Mmp9*.
Carvan et al. (2017)	Zebrafish		Transgenerational (F0 and F2), F0 generation embryos were exposed to 1, 3, 10, 30, and 100 nM for 24 h	291 DMRs in F0 sperm and 617 DMRs in F2 sperm.
Go et al. (2021)	Human (*Homo sapiens*)	LUHMES cell-line	Chronic (6 days); 1 nM	Increased global DNA methylation. Increased protein levels of DNMT1, DNMT3A, and DNMT3B. Decreased levels of AcH3 and AcH3K14. Increased protein levels of H3K27me3, HDAC3, and HDAC6.
Go et al. (2021)	Mouse	C57BL/6 J	Gestational (from embryonic day 12 to embryonic day 14); 3 mg/kg/day	Increased global DNA methylation and DNMT1 protein levels. Decreased AcH3 and AcH3K14, and increased HDAC3 and HDAC6 protein levels.
Pan et al. (2022)	Mouse	HT22 cell-line	Acute (24 h); 1, 2, or 4 μM	Increased protein levels of DNMT3A, DNMT3B, and DNMT1.
Cediel-Ulloa et al. (2022)	Human	SH-SY5Y cell-line	Chronic (6 days); 8 and 40 nM	Increased *MED31* DNA methylation.
Pan et al. (2022)	Mouse	ICR	Gestational (from embryonic day 0 to day 18); 4, 8 or 12 mg/L every other day	Increased global DNA methylation at 8 mg/L. Increased DNMT1, DNMT3A, and DNMT3B protein levels.
Histone modifications				
Choi and Simpkins (1986)	Mouse	Primary fetal astrocytes	Acute (6 h); 10 μM	Decreased H3 accessibility.
Guida et al. (2016)	Human	SH-SY5Y cell-line	Acute (24 h); 1 μM	Decreased H4 acetylation.
Guida et al. (2016)	Mouse	C57/BL6, adult male	Chronic (10 days); 10 mg/Kg/day	Decreased H4 acetylation.
Guida et al. (2017)	Human	SH-SY5Y cell-line	Acute (12 and 24 h); 1 μM	Increased HDAC4 protein and gene expression.
Rudgalvyte et al. (2017)	Roundworm (*Caenorhabditis elegans*)		Chronic (from larval stage L1 to larval stage L4 or for 96 h at L4); 10 μM	Changes in H3K4me3 of 1975 genes. Increased H3K4me3 at *lpr-5*, *gst-5*, *gst-38*, *dpy-7*, and *atf-6*.
Go et al. (2018)	Human	LUHMES cell-line	Chronic (6 days); 1 nM	Increased H3K27me3 at the promoter of tyrosine hydroxylase.
miRNA				
Rudgalvyte et al. (2013)	Roundworm		Chronic (from embryonic to L4 larval stage); 10 μM	Decreased expression of *miR-37–3p*, *miR-41–5p*, *miR-70–3p*, and *miR-75–3p*.
Pallocca et al. (2013)	Human	NTERA-2 clone D1 cell-line	Chronic (5 weeks); 400 nM	Increased expression of *miR-302b*, *miR367*, *miR-372*, *miR-141*, and *miR-196b*.
Wang et al. (2016)	Human	ReNcell CX cell-line	Acute (24 h); 10 and 50 nM	Decreased expression of *miR-1285*, *miR-30d*, and *miR-25*.
Hu et al. (2017)	Zebrafish	Embryos 48 hpf	Acute (24 h); 0.01 mg/mL	61 differentially expressed miRNAs. Increased expression of *dre-miR-375* and *dre-miR-206*. Decreased expression of *dre-miR-7147* and *dre-miR-26a*.
Guida et al. (2018)	Rat	Primary cortical neurons	Acute (6, 12, and 24 h); 1 μM	Decreased expression of *miRNA-206*.
Nielsen et al. (2021)	Roundworm		Acute (30 min) exposure of L1 worms; 1, 10, or 20 μM	Increased mortality in *nrde-2* and *pash-1* mutant worms.
Wang et al. (2022)	Mouse	Primary hippocampal neurons	Acute (24 h); 0.625, 1.25, 2.5, and 5 μM	Decreased expression of *miR-9–5p*.
Wang et al. (2022)	Rat		Gestational (from embryonic day 15 to postnatal day 21); 1.2 mg/kg/day	Decreased *miR-9–5p* levels.

hippocampus (Onishchenko et al., 2008). Here, DNA methylation changes were accompanied by alterations in histone modifications (see below), decreased *Bdnf* expression, and depression-like behaviour in the mice (Onishchenko et al., 2008), which is in line with Bdnf's role in neurodevelopment and behaviour (Homberg et al., 2014). Thus, this study provides some evidence that the MeHg-induced epigenetic changes may have functional consequences in the brain.

Other studies have reported transgenerational epigenetic effects of MeHg, i.e., transmission of epigenetic markers from parent to child. For example, Olsvik et al. (2014) exposed adult female (F0) zebrafish to 10 mg/kg MeHg for 47 days. Although no differences in global methylation levels were seen in the F0 females or the subsequent F1 and F2 generations (3-day-old embryos), genome-wide analyses revealed locus-specific differential methylation in the livers of F0 females and in those of F1 and F2 embryos. Exposure of F0 females to MeHg led to hypomethylation of 49 genes, of which 41 were protein-coding genes and two were miRNAs (Olsvik et al., 2014). These genes were associated with processes related to neurological systems ($n = 8$), activity of gap junctions ($n = 2$), sensory perception ($n = 5$), and other general cellular and biological processes. In F1 embryos, 32 genes were differentially methylated following MeHg exposure of the F0 females, and these genes were associated with neuroactive ligand-receptor interaction, glycosphingolipid biosynthesis, GTP-Rho binding, metabolic processes, hypotension, G-protein receptor signalling and activity, and other cellular signalling processes. In F2 embryos, exposure of the F0 females to MeHg resulted in hypermethylation of one gene. There was no overlap in differentially methylated genes between F0, F1, and F2 generations, but four genes were common between F0 and F1 (*CR556710.1*, *FP102191.1*, *taar20p*, and *KCNJ2*) (Olsvik et al., 2014). As no phenotypic transgenerational effects were reported in this study, functional implications of these changes remain unclear.

In another transgenerational study in zebrafish, sperm from adult F0 and F2 generations that had been exposed to 30 nM MeHg for 24 h in their embryonic state showed differential methylation in a genome-wide analysis: 291 differentially methylated regions (DMRs) within or close to genes mainly involved in translation were identified in F0 sperm (Carvan et al., 2017). F2 sperm contained 617 DMRs associated with genes involved in neuroactive ligand-receptor interaction and actin-cytoskeleton pathways. Of these DMRs, 135 overlapped between the F0 and F2 generations. The study provided a well-described evaluation of the MeHg levels accumulated in F0 tissue of exposed (~ 20 ppb to ~ 3000 ppb) and control (~ 5 ppb) animals, information commonly missing in other studies but of high importance for result comparisons.

3.2. Epidemiological studies

Most epidemiological studies (Table 2) addressing MeHg and epigenetic changes have evaluated the associations of MeHg exposure during gestation and DNA methylation, with a few studies evaluating global methylation changes in relation to MeHg. DNA methylation at 5mC sites can undergo oxidation to form 5-hydroxymethylcytosine (5hmC). Regulation of gene expression by 5hmC is independent of 5mC and is important during embryogenesis, particularly in neurogenesis (Etchegaray et al., 2015). In a U.S. mother–child cohort, Cardenas et al. (2017a) found that prenatal MeHg exposure, measured as Hg in maternal red blood cells, was associated with a lower percentage of 5hmC in the genome and a corresponding increase in the ratio of the percentage of 5mC to the percentage of 5hmC in cord blood. This association was persistent in early (2.9–4.9 years) but not mid-childhood (6.7–10.5 years) blood. This cord blood finding by Cardenas et al. (2017a) was not supported by a more recent study of a Norwegian mother–child cohort (Weyde et al., 2020). They analysed associations between mid-gestational levels of blood Hg and global 5mC and 5hmC levels in cord blood. An increase in maternal blood Hg level was associated with a reduction in 5mC in newborns; however, increasing gestational Hg was not associated with 5hmC. It should be noted that the U.S. study base was approximately half the size of the Norwegian study and the lack of confirmation of 5hmC changes in the latter study is therefore not likely due to limited statistical power.

Candidate gene studies have focused on genes related to the nervous system. In 7-year-old Seychellois children exposed to MeHg *in utero* (mean maternal hair concentration during pregnancy = 4.70 ppm), associations between prenatal MeHg exposure and DNA methylation were found for one CpG site in *Glutamate receptor subunit NR2B* (*GRIN2B*) and two CpG sites in the glucocorticoid receptor gene *NR3C1* out of 14 CpG sites examined (Cediel Ulloa et al., 2021). Higher prenatal MeHg was associated with increased methylation for each CpG site. Two out of three CpG sites associated with MeHg were located in transcription factor binding sites, and the methylation changes observed were predicted to reduce gene expression. One *NR3C1* CpG and one *BDNF* CpG showed higher methylation in boys but not in girls exposed to higher prenatal MeHg (Cediel Ulloa et al., 2021). The NR3C1 receptor is a crucial factor in stress responses in the brain via its regulation of the hypothalamic–pituitary adrenal axis. Lower levels of the glucocorticoid receptor from increased DNA methylation might result in lower responsiveness to cortisol and other glucocorticoids, whose functions include decreasing inflammation and regulating stress responses (Binder, 2009; Rhen and Cidlowski, 2005). Still, it is unclear if the Hg-related DNA methylation may have any effect on stress response, as the DNA methylation was measured in saliva and not in the brain. This is not only an issue for this study, but for most epidemiological studies where epigenetic marks are measured in proxy tissues.

Prenatal MeHg levels and DNA methylation of *NR3C1* were also evaluated in a study of placentas from a U.S. mother–child cohort (Appleton et al., 2017). Higher levels of Hg in infant toenails (average of 0.17 μg/g in the highest tertile), a biomarker for prenatal MeHg exposure, were associated with an increase in placental *NR3C1* methylation. Maccani et al. (2015) analysed the same U.S. mother–child cohort and found associations between prenatal Hg exposure and DNA methylation by measuring genome-wide DNA methylation of specific CpGs (so called EWAS) in 192 placenta samples, as well as neurobehavioral outcomes in newborns using the NICU Network Neurobehavioral Scales (NNNS). Six loci near the *EMID2* gene (official gene name in NCBI: Collagen type XXBI alpha 1 chain, *COL26A1*) were hypomethylated in 16 placentas from infants with high-risk NNNS profiles, associated with more behavioural problems later in life. The *EMID2* methylation was also correlated to *EMID2* gene expression. While the MeHg exposure was associated with lower DNA methylation of EMID2 in utero, higher DNA methylation of EMID2 was associated with NNNS. It is therefore unclear if MeHg could cause different neurobehavioral outcomes in newborns related to methylation in *EMID2/ COL26A1*. It should be noted that there was no significant association between MeHg with *NR3C1* in this study. However, the study was rather small and the study may have been underpowered.

In a EWAS study of 138 U.S. children, prenatal MeHg measured in maternal toenails, was associated with differential methylation of 9 ($p < 0.0001$) out of 348,569 CpG loci analyzed in cord blood (Cardenas et al., 2015). However, the differential methylation was not significant after controlling for multiple adjustment. In another EWAS study of 141 U.S. children, low-level exposure to total Hg (median 1.4 μg/L) or MeHg, measured in cord blood, was associated with differential (inverse) methylation in cord blood of a region of the *Transcription Elongation Factor A (SII) N-Terminal and Central Domain Containing 2* (*TCEANC2*) gene, a gene with unknown function. This MeHg-related DNA methylation signal was supported in an independent sample. No association was found between *TCEANC2* methylation and gene expression and the functional consequences of lower methylation of *TCEANC2* in relation to MeHg are yet unknown (Bakulski et al., 2015).

In a small EWAS study of a mother-child cohort from the Faroe Islands, Hg concentrations in cord blood and maternal hair were investigated (Leung et al., 2018) and concentrations in maternal hair were associated with differential DNA methylation of five CpGs (no

Table 2
Epidemiological studies of exposure to methylmercury (MeHg) and DNA methylation (DNAm) or miRNA.

Reference; country, study design, nr of participants	MeHg exposure	Gene (protein name) function	CpG site	Associations between Hg and DNAm /adjustments
DNA methylation				
Goodrich et al. (2013); USA Dental professionals + candidate gene approach, N = 131	Hair Hg geometric mean (95 % CI)= 0.37 (0.31–0.44) μg/g	*SEPP1* (Selenoprotein P, plasma, 1) transports selenium to extra-hepatic tissues, extracellular antioxidant	4 CpG	Higher Hg concentrations associated with lower DNAm in males.
Cardenas et al. (2015); USA Mother-child cohort + EWAS, N = 138	Hg in maternal toenails: median Hg= 0.07 μg/g (range 0.001–1.44)	No genes reported	9 CpG differentially methylated; p < 0.0001	Increased methylation (85 %) in north side shore regions in samples with the lowest p-values (n = 20). /Adjusted for maternal age at delivery, and infant sex, estimated white blood cell composition, CD8 T-cells, CD4 T-cells, NK cells, B-cells, monocytes, and granulocytes.
Bakulski et al. (2015); USA Mother-child cohort + EWAS, N = 141, replication sample N = 77	Hg in cord blood median (IQR)= 1.4 (1.0–2.0) μg/L MeHg= 0.9 (0.6–1.6)	*TCEANC2* (Transcription Elongation Factor A N-terminal and Central Domain containing 2), predicted to be involved in transcription	7 CpG in a differentially methylated region	Higher total Hg and MeHg concentrations were associated with higher DNAm both in the larger and the replication samples
Maccani et al. (2015); USA Mother-child-cohort + EWAS, N = 192 placentas	Hg in infant toenail: low tertile 0.005–0.031 μg/g, medium tertile 0.032–0.076 μg/g, high tertile 0.077–0.425 μg/g	*COL26A1* (*EMID2* in paper; Collagen type xxvi α-1 chain) protein contains an emilin domain and two collagen stretches	cg14048874 cg13267931cg27179533cg14874750cg23424003cg27528510	High Hg tertile associated with higher placenta DNAm. Hypomethylation in placentas with high-risk neurobehavioural profile (NNNS) n = 16. /Adjusted for maternal age, birth weight percentile, delivery method, and infant gender
		CPLX1 (Complexin 1), cytosolic protein that functions in synaptic vesicle exocytosis, proteins bind syntaxin, a part of SNAP receptor	cg17128947	High Hg tertile associated with lower placenta DNAm.
		TTC23 (Tetratriopeptide repeat domain 23)	cg25385940	High Hg tertile associated with lower placenta DNAm.
Appleton et al. (2017); USA Mother-child cohort + candidate gene approach, n = 222 placenta	Hg in toenailsmean (SD)= 0.07 (0.10) μg/g Hg lowest tertile: 0.01, middle tertile: 0.03, high tertile: 0.17 μg/g	*NR3C1* (Nuclear receptor subfamily 3 group C member 1), glucocorticoid receptor, a regulator of stress responses, inflammatory responses, cellular proliferation, and differentiation	13 CpG in exon 1 F	Higher Hg concentrations associated with increased placental DNAm. /Maternal age, ethnicity, education, pre-pregnancy BMI, prenatal tobacco use, prenatal depression, infant gender, and birthweight percentile
Cardenas et al. (2017a); USA Mother-child cohort, n = 306 cord blood, n = 68 early childhood, n = 260 midchildhood	2nd trimester red blood cell mercury (RBC-Hg), median= 3.32 μg/g IQR= 3.29	Global DNAm via 5-hydroxy-methylcytosine (5hmC) and 5-methylcytosine (5mC)	n.a.	Change in ratio of %5mC to %−5hmC for a doubling in Hg concentration was higher at birth, early, and attenuated at midchildhood. /Maternal education, age at enrollment, marital status, vitamin B-12, betaine intake, fish consumption, and child ethnicity, sex, gestational age, and birth weight for gestational age z-scores
Cardenas et al. (2017b); USA Mother-child cohort + EWAS, n = 321 cord blood, n = 75 early childhood, n = 291 mid childhood	Hg in maternal red blood cells in 2nd trimester of pregnancy: 3.8 ng/g,early childhood: 4.2 ng/g, mid-childhood: 4.0 ng/g	*PON1* (Paraoxonase 1) antioxidant protein that provides protection to with high density lipoprotein particles against oxidation	cg07404485 cg19678392 cg01874867 cg05342682 cg20119798 cg04155289 cg17330251 cg04871131 cg21856205	Among males, Hg concentrations were associated with lower regional cord blood DNAm that persisted in early childhood and was attenuated in mid-childhood blood. Cord blood methylation at the *PON1* locus predicted lower

(*continued on next page*)

Table 2 (*continued*)

Reference; country, study design, nr of participants	MeHg exposure	Gene (protein name) function	CpG site	Associations between Hg and DNAm /adjustments
				PPVT scores (Peabody Picture Vocabulary Test) measured during early childhood. /Maternal age, ethnicity, fish intake, pre-pregnancy BMI, smoking, parity and college education, gestational age, sex (if not stratified), and estimated nucleated cell types in cord blood.
		WBP11P1 (WW Domain Binding Protein 11 Pseudogene 1)	cg13340705	Higher Hg concentrations associated with lower DNAm in cord blood. 10 % higher DNAm associated with lower estimated PPVT score in males and females, and lower estimated WRAVMA (Wide Range Assessment of Visual Motor Abilities) in females.
		TOR4A (Torsin Family 4 Member A) predicted to enable ATP binding activity, and to be active in endoplasmatic reticulum lumen and nuclear envelope	cg13416866	Higher Hg concentrations associated with higher DNA methylation in cord blood and mid-childhood.
Leung et al. (2018), Faroese, Mother-child cohort + EWAS, N = 72 (only 51 included in the analysis)	Cord blood Hg: Mean (SD)= 19.01 (8.14) μg/L Maternal hair Hg: 3.53 (1.68) μg/g	n.a.	5 CpGs	Higher Hg concentrations in maternal hair were associated with different DNAm in cord blood. /Adjusted for alcohol consumption, maternal age, gestational age, slide number
Nwanaji-Enwerem et al. (2020); USA Cohort study + EWAS, N = 48	Hg in 24-h urine: Mean (SD)= 1.6 ng/mL (1.5)	PhenoAge, epigenetic clock of DNA methylation in multiple genes, a predictor of lifespan, physical functioning, and healthspan	513 CpG	5-metal exposure mixture (As, Cd, Hg, Pb, and Mn) associated with increase in PhenoAge. / Chronological age, season of visit, GFR, BMI, alcohol intake, pack-years, smoking status, education, white blood cell proportion, and in sensitivity analysis diabetes, hypertension, and ischemic heart disease.
Nishiawa-Jotaki et al., 2021; Japan Mother-child cohort + EWAS, N = 67 (male n = 27)	Hg in cord serum: Median (IQR) in boys = 0.900 (0.645) ng/g, girls = 0.620 (0.473)	*HDHD1* (Haloacid dehalogenase-like hydrolase domain-containing protein 1) associated with Crohn's disease and X-linked ichthyosis, may be involved in RNA processing and turnover	cg02027844; Intron 4	Higher Hg concentrations associated with higher DNAm of cg02027844 in males but not in females. /Adjusted for maternal age, birth weight, gestational age, Se concentration in cord serum, endothelial ratio and epithelial ratio.
Weyde et al. (2021); Norway Parent-child cohort N (newborn children)= 631	Hg in maternal whole blood in week 17–18 of pregnancy: Mean (SD)= 1.47 (1.04)	Global DNAm via 5-hydroxy-methylcytosine (5hmC) and 5-methylcytosine (5mC)	n.a.	Hg concentrations were negatively associated with child 5mC. No associations with 5hmC. /Maternal intake of folate, iodine, vitamin B12 and seafood during pregnancy, smoking during pregnancy, maternal education, age, parity and sex of the child
Cediel Ulloa et al. (2021); Seychelles Mother-child cohort + candidate gene approach, N = 406	Maternal hair Hg: mean (SD)= 4.70 (4.19) ppm	*NR3C1* (Nuclear receptor subfamily 3 group C member 1), glucocorticoid receptor, a regulator of stress and inflammatory responses, cellular proliferation, and differentiation	5 CpG in exon 1 F	Higher Hg concentrations associated with higher DNAm for two CpG. For one CpG, there was a sex interaction: higher hair Hg concentrations were associated with higher DNAm in boys, but not in

(*continued on next page*)

Table 2 (*continued*)

Reference; country, study design, nr of participants	MeHg exposure	Gene (protein name) function	CpG site	Associations between Hg and DNAm /adjustments
				girls. /Maternal age at delivery, BMI at 20 months, Hollingshead Socioeconomic Status, sex of the child, birth weight, and gestational age
		GRIN2B (Glutamate receptor subunit NR2B), NR2B subunit of the N-methyl-D-aspartate receptor (NMDAR), a receptor for the excitatory neurotransmitter glutamate, important for the regulation of neuronal morphology, learning and memory	4 CpG in predicted intergenic promoter region	Higher Hg concentrations associated with higher DNAm for one CpG.
		BDNF (Brain-derived neurotropic factor) plays a role in neural development, nerve cell survival and synaptic plasticity	5 CpG in intron 3	Sex interaction for prenatal hair Hg where higher hair Hg was significantly associated with higher DNAm in boys but not girls.
Lozano et al. (2022); multiple countries Mother-child cohort + EWAS N = 1462 from Spain, Korea, USA, Japan, UK, Greece, Norway	Cord blood geometric mean range 2.01–9.30 μg/L	*GGH* (Gamma-glutamyl hydrolase) is involved in the folic acid and glutamate metabolism	cg02212000	Higher prenatal Hg concentrations associated with lower DNAm in child blood at 7 years, only in model excluding fish consumption. /Adjusted for maternal age, parity, education, smoking status during pregnancy), maternal fish consumption (mean daily servings), child's sex, and cellular heterogeneity.
		MED31 (Mediator complex subunit 31) enables transcription coregulator activity and ubiquitin protein ligase activity, predicted to be involved in regulation of transcription by RNA polymerase II	cg24184221	Higher prenatal Hg concentrations nominally associated with higher DNAm in cord blood blood
			cg15288800	Higher prenatal Hg concentrations nominally associated with higher DNAm in child blood
		GRK1 (G protein-coupled receptor kinase 1), phosphorylates rhodopsin and initiates its deactivation	cg12204245	Higher prenatal Hg concentrations associated with higher DNAm in child blood at 7 years
		PWWP2B (PWWP domain containing 2B)	cg17452301	Higher prenatal Hg concentrations associated with higher DNAm in child blood with adjustment for fish consumption, no persistence between cord and child blood
		DNAH7 (Dynein axonemal heavy chain 7), component of inner dynein arm of ciliary axonemes	cg19374305	Higher prenatal Hg concentrations associated with higher DNAm in child blood with adjustment for fish consumption no persistence between cord and child blood
		CTNND2 (Catenin delta 2), protein implicated in brain and eye development and cancer formation	cg10555307	Higher prenatal Hg concentrations associated with higher DNAm in child blood with adjustment for fish consumption
Aung et al. (2022); USA Mother-child cohort + EWAS in mothers, N = 97	Hg maternal blood: geometric mean	*SPTBN2* (Spectrin beta, non-erythrocytic 2), protein regulates the	cg17112108	Higher Hg concentration associated with higher DNAm. /Adjusted for cell

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Table 2 (*continued*)

Reference; country, study design, nr of participants	MeHg exposure	Gene (protein name) function	CpG site	Associations between Hg and DNAm /adjustments
	(GSD) = 0.60 (2.15) μg/L	glutamate signaling pathway, mutations cause progressive locomotor incoordination		type composition, maternal ethnicity, maternal education, and household income
		TNXB (Tenascin XB), extracellular matrix glycoprotein with antiadhesive effects, may function in matrix maturation during wound healing	cg04539172	Higher Hg concentration associated with higher DNAm
		IL11 (Interleukin 11), cytokine that may stimulate T-cell-dependent development of immunoglobulin-producing B cells,	cg01975672	Higher Hg concentration associated with lower DNAm
		n.a.	cg03782771	Higher Hg concentration associated with higher DNAm
		n.a.	cg02128086	Higher Hg concentration associated with higher DNAm
		DMKN (Dermokine), upregulated in inflammatory diseases	cg15817481	Higher Hg concentration associated with lower DNAm
		n.a.	cg05690088	Higher Hg concentration associated with lower DNAm
		n.a.	cg13954206	Higher Hg concentration associated with lower DNAm
		POU4F3 (POU class 4 homeobox 3), transcription factor that plays an important role in control of cell identity	cg26932154	Higher Hg concentration associated with lower DNAm
		n.a.	cg04023226	Higher Hg concentration associated with higher DNAm
miRNA				
Li et al. (2015); USA, N = 43	Hg in placenta: median (min-max)= 0.8 (0.3–3.3) pg/g	Let-7 family, miR-151–5p, miR-193b, miR-1975, miR-423–5p, miR-520d-3p, miR-96, miR-526aC, miR-518d-5pC, and miR-520c-5p	n.a.	Above median Hg concentration in placenta was associated with a lower expression of multiple miRNAs in cervical smears.
Sanders et al. (2015), Mexico, N = 40,	Hg in toenails: mean (SD)= 0.17 (0.09) μg/g	miR-205–5p, miR-125b-5p, let-7b-5p, miR-200c, miR-342, miR-203, let-7a-5p, miR-24–3p, miR-22–3p, miR-23b-3p, miR-375, miR-23a, miR-210, miR-200b-3p, miR-99a-5p, miR-21–5p	n.a.	Higher Hg concentration in toenails was associated with lower expression of multiple microRNAs in cervix.
Kupsco et al. (2022), Faroe Islands, N = 333	Hair Hg: geometric mean (GSD) 2.25 (2.31) μg/g	*miR-200b-3p, miR-664a-3p, miR-6738–5p, miR-429, miR-1236–5p, miR-4464, miR-30b-5p*	n.a.	The authors mention that Hg in hair was associated with 13 miRNAs in breast milk but only mentions 7 in the text and no individual models are shown.
Longo et al. (2022), Italy, N = 68	Serum Hg during the last trimester of pregnancy: median (IQR)= 0.57 (0.20–0.85) μg/L	*miR-30b*, *miR-223*, *Let-7a*	n.a.	Higher Hg concentration in serum was associated with lower expression of *miR-30b* in serum.

n.a. not available

information on CpG position in the genome was provided). No significant associations between Hg in cord blood and DNA methylation were found.

In 2021, an EWAS between prenatal Hg exposure, measured as Hg concentrations in cord serum, and DNA methylation in cord tissue was conducted in a small mother–child cohort from Japan (Nishizawa-Jotaki et al., 2021). Methylation of cg02027844 in the *Hydrolase domain-containing protein 1* (*HDHD1*) gene was significantly correlated with higher Hg concentrations in cord serum of males, but not females. *HDHD1* on chromosome X has been associated with X-linked intellectual

disability (Labonne et al., 2020). HDHD1 is a phosphatase specifically involved in dephosphorylation of a modified nucleotide present in RNA (Preumont et al., 2010).

There are only a few studies on the persistence of the effects of prenatal MeHg exposure on DNA methylation during childhood. A U.S. mother–child study reported an association between low-level prenatal MeHg exposure (maternal erythrocyte Hg = 3.8 ng/g, representing Hg concentration in separated erythrocytes from whole blood) and reduced methylation of a differentially methylated region in *Paraoxonase 1* (*PON1*), a gene involved in Phase I biotransformation and fatty acid metabolism, in cord blood of boys (Cardenas et al., 2017b). This reduction in methylation persisted throughout childhood and was also associated with poorer performance in one of the neurodevelopmental outcomes studied, suggesting that DNA methylation may act as a long-term mediator of the effects of MeHg exposure. A strength of the study was that it assessed the stability of DNA methylation in *PON1* over time, which was surprisingly stable in a smaller subset of the study participants.

Lozano et al. (2022) examined in the largest study so far associations between prenatal MeHg exposure and levels of DNA methylation (373, 251 CpGs) in cord blood using meta-analysis of seven independent mother–child studies, as well as persistence of those relationships in blood of 7- to 8-year-old children. Differential methylation of cord blood DNA in relation to prenatal MeHg exposure was found in six genes, and persistence in children was identified in one: *MED31*. This gene is involved in RNA Polymerase II transcription and lipid metabolism. Cg12204245 displayed 0.2 % higher DNA methylation when total Hg was doubled, and when adjusting for maternal fish consumption. Lower methylation of cg02212000 in the *Gamma-glutamyl hydrolase* (*GGH*) gene as well as hypermethylation of cg12204245 in the *G protein-coupled receptor kinase 1* (*GRK1*) gene was also found in children. The other three genes showed less consistent results between cohorts, age groups and analysis with and without adjustment for fish. Some experimental support for these findings were reported in Cediel Ulloa et al., (2022).

In an EWAS evaluating the influence of MeHg on the *maternal* epigenome in a U.S. mother–child cohort, no significant associations were observed after correction for multiple comparisons between maternal Hg concentrations and blood DNA methylation (Aung et al., 2021). With increasing Hg concentrations hypermethylation at several CpGs were identified, e.g., near the *Spectrin beta, non-erythrocytic 2* (*SPTBN2*), as well as hypomethylation at several CpGs.

Most studies of MeHg exposure and DNA methylation so far have evaluated associations with gestational exposure in newborns and children. One of the few exceptions is a study by Goodrich et al. (2013) that investigated the association between Hg in hair (indicative of MeHg) and in urine (inorganic Hg) and global DNA methylation by examining methylation of *LINE1* transposon elements, *DNA methyltransferase 1* (*DNMT1*), *Selenoprotein W* (*SEPW1*), and *Selenoprotein P (SEPP1*) in 131 dental professionals. The study revealed a trend of *SEPP1* hypomethylation with increasing hair Hg levels, particularly in male dental professionals. No association was found between Hg in urine and DNA methylation, suggesting alterations in *SEPP1* methylation to be specific for MeHg.

Another exception is a longitudinal cohort study of ageing, examining the relationship between Hg in urine of elderly men over a 24-h period and the methylation-based ageing parameters DNAmAge, PhenoAge, and GrimAge (Nwanaji-Enwerem et al., 2020). Urine Hg (mean 1.6 ng/mL ± 1.5 SD), in combination with arsenic, cadmium, manganese, and lead, was linked to PhenoAge. A one-unit increase in the cumulative five-metal exposure-mix with each metal at its 70th percentile was associated with a 2.53-year increase in PhenoAge ($p < 0.05$). At its 90th percentile, a one-unit increase was associated with a 6.10-year increase in the DNA-methylation-based biological age. However, Hg in urine mainly reflects inorganic Hg, and MeHg may have no or different associations with ageing.

4. MeHg and histone modifications

4.1. Animal and in vitro studies

Several experimental studies report effects of MeHg on enzymes involved in histone modifications and on global changes in histone modifications (Table 1). An early study of mouse primary astrocytes showed that histone H3 was less accessible after exposure to 10 μM MeHg for 6 h. The authors suggested that MeHg may bind directly to the cysteine residue in H3 and that this change could be of importance in gene regulation (Choi and Simpkins, 1986). Nonetheless, the results were assessed with the use of a fluorescent probe (N-(3-pyrene)maleimide) and are a reflection of changes in fluorescence intensities, which makes the results difficult to compare to newer, more accurate techniques.

Exposure to 1 μM MeHg (MeHg chloride) for 24 h increased protein and mRNA levels of histone deacetylase 4 (HDAC4, an enzyme that catalyzes the removal of acetyl groups from histones, which in turn influence the accessibility of DNA for gene expression) and decreased global H4 acetylation in human SH-SY5Y cells (Guida et al., 2016). However, this MeHg concentration induced cytotoxicity, with a loss of 50 % viable cells (Guida et al., 2016); thus, it cannot be concluded whether these are direct effects of the exposure or indirect effects resulting from cell death. However, the authors strengthened their in vitro findings with investigations in adult male C57/BL6 mice exposed to MeHg at 10 mg/kg/day for 10 days, which showed decreased H4 acetylation in the granular layer of the cerebellum (Guida et al., 2016). These epigenetic changes were linked to cellular endpoints indicating apoptotic cell death induced by MeHg, and mediated by the RE-1–silencing transcription factor (REST), a transcriptional repressor. MeHg effects on cell viability were rescued by transfection with siRNA against REST, and by co-exposure of MeHg with the pan-histone deacetylase inhibitor trichostatin-A, thus providing evidence that reduction of global H4 acetylation could underlie MeHg's cytotoxic effects.

HDAC expression and global acetylation are also affected by MeHg in chronic exposure scenarios. LUHMES cells, i.e. human dopaminergic neurons, exposed to 1 nM MeHg for 6 days showed increased protein levels of HDAC3 and HDAC6 and decreased global H3 and H3K14 acetylation (Go et al., 2021). These effects were validated in vivo, where foetuses of mice exposed gestationally to MeHg at 3 mg/kg/day showed an increase in HDAC3 and HDAC6 in the cerebral cortex, concomitant with decreased global levels of H3 and H3K14 acetylation (Go et al., 2021). As for the DNA methylation changes described in the previous section, functional implications of the epigenetic changes were linked to cellular outcomes (decreased neurite length), and consistently with the experiments reported in the previous section, co-exposure of MeHg and HDAC inhibitor rescued the effect on neurite length, reinforcing the link between epigenetic changes and cellular neurodevelopmental outcomes.

In LUHMES cells, a global increase in methylation of histone 3 at H3K27me3 was also observed, as well as increased H3K27me3 at the promoter of the tyrosine hydroxylase gene, along with decreased protein levels of the enzyme (Go et al., 2018). Tyrosine hydroxylase is the rate-limiting enzyme in dopamine and other catecholamine synthesis, hence this study suggests an effect of MeHg on dopamine synthesis via an epigenetic mechanism. However, this would need to be confirmed with functional assays.

Locus-specific effects have also been identified in mice developmentally exposed to MeHg at 0.5 mg/kg/day. In 12-month-old males, this exposure decreased H3 acetylation and increased H3K27me at promoter IV of *Bdnf*. As mentioned above, the epigenetic changes were accompanied by decreased *Bdnf* expression and depression-like behaviour (Onishchenko et al., 2008). Lastly, an epigenome-wide study of chronic MeHg exposure in *Caenorhabditis elegans* (10 μM MeHg from larval stage L1 to larval stage L4) identified changes in H3K4me3 in

1975 genes, 1467 with increased and 508 with decreased H3K4me3. Of these, 50 genes were both differentially methylated and differentially expressed (43 upregulated and 7 downregulated); targeted analyses of *lpr-5*, *gst-5*, *gst-38*, *dpy-7*, and *atf-6* confirmed previous results (Rudgalvyte et al., 2017). Additionally, Increased H3K4me3 in *lpr-5*, *gst-5*, *gst-38*, *dpy-7*, and *atf-6* was also demonstrated in *C. elegans* exposed to 10 μM MeHg for 96 h during embryogenesis and evaluated once the L4 stage was reached; however, changes in gene expression were only evident for *lpr-5* (Rudgalvyte et al., 2017). Although only one concentration of MeHg was studied limiting comparisons with other available data, the functional consequences of lpr-5 and dpy-7 knockdowns were addressed showing increased lethality upon exposure.

To our knowledge, there are no epidemiological studies on histone changes in relation to MeHg exposure.

5. MeHg and microRNAs

5.1. Animal and in vitro studies

Numerous experimental studies indicate that exposure to MeHg has the potential to interfere with normal miRNA expression (Table 1). For instance, chronic exposure of human neuronal NT2 cells to 400 nM MeHg for 5 weeks increased expression of *miR-302b*, *miR367*, and *miR-372*, which are involved in maintenance of pluripotency; *miR-141*, associated with oxidative stress response and cell differentiation; and *miR-196b*, a regulator of HOX genes (Pallocca et al., 2013). However, this MeHg concentration produced about 30 % reduction in cell viability after 5 weeks of exposure (Pallocca et al., 2013); thus, the miRNA changes observed could be indirect effects resulting from cytotoxicity.

Acute exposure to low, non-cytotoxic concentrations of MeHg (10 nM) for 24 h led to decreased expression of *miR-30d* and *miR-25*, targeting the tumour suppressor p53, in immortalized human embryonic neural progenitor cells (ReNcell CX cells) (Wang et al., 2016). At a higher MeHg concentration (50 nM), these miRNAs and *miR-1285* were also downregulated, but decreased cell proliferation and viability were observed. These findings were accompanied by increased ROS production, increased mitochondrial DNA copy number, and upregulation of the cell cycle genes *CDKN1A*, *CDKN2A*, and *TP53* (Wang et al., 2016). In another acute setting, exposure of rat primary cortical neurons to 1 μM MeHg for 24 h downregulated the expression of *miR-206* and increased the release of lactate dehydrogenase, indicating neuronal death (Guida et al., 2018). In the same study, *miR-206* transfection decreased the cytotoxicity induced by MeHg and promoted protein and gene expression of *Bdnf*, thereby providing a functional link between MeHg-induced *miR-206* down-regulation and cytotoxicity.

Primary fetal mouse hippocampal neurons showed decreased expression of *miR-9–5p* after MeHg exposure at concentrations ranging from 625 nM to 5 μM for 24 h (Wang et al., 2022). These findings were accompanied by increased mitochondrial membrane polarization, decreased neurite length, reduced levels of the synaptic proteins synapsin-1(SYN) and postsynaptic density protein-95 (PSD-95), and increased protein levels of *Forkhead box protein P2* (FOXP2), a transcription factor important for language development and reported to function as synapse regulator. The effects of MeHg were rescued by *miR-9–5p* transfection (Wang et al., 2022), providing a functional link between *miR-9–5p* expression and cellular effects of MeHg exposure. In the same study, a reduction in *miR-9–5p* levels was found in the hippocampus of PND60 rats gestationally exposed to MeHg at 1.2 mg/kg/day, accompanied by other molecular changes such as reduction of synaptic protein levels and alterations in dendritic spine morphology, thus strengthening the in vitro findings.

Overall, these studies provide further evidence of the involvement of epigenetic marks, in the form of miRNAs, on (developmental) neurotoxicity, which is in line with the known toxicological mechanisms of MeHg in the central nervous system (e.g. oxidative stress, impairment of neurogenesis, and cell death) (Martins et al., 2021).

MeHg-related changes in miRNAs have not only been shown in rats but also in other in vivo models. A single exposure of zebrafish embryos to 0.01 mg/mL MeHg at 48 h post fertilization resulted in 61 differentially expressed miRNAs. The miRNAs *miR-206*, *dre-miR-7147*, *dre-miR-26a*, and *dre-miR-37*23 targeted 23 differentially expressed genes involved in the cardiac muscle contraction pathway. Targeted analysis revealed decreased expression of *dre-miR-7147* and *dre-miR-26a* and increased expression of *dre-miR-375* and *dre-miR-206* in embryos exposed to MeHg (Hu et al., 2017). However, cardiotoxicity was not measured and thus no conclusion on the functional implications of these changes can be drawn.

MeHg-induced mortality was shown to be dependent on miRNA expression by comparing wild-type *C. elegans* with mutant animals displaying decreased miRNA expression, resulting from mutations in genes that are essential for gene silencing and miRNA maturation (Nielsen et al., 2021). Mortality was induced at lower MeHg concentrations (LD50) in the mutant than in the wild-type strain following acute exposure to 1–20 μM MeHg for 30 min at the L1 stage. Furthermore, the mutant worms showed metabolic disturbances (increased total triglycerides, fat storage, and food consumption) and increased mitochondrial toxicity in response to MeHg exposure, whereas the wild-type animals did not (Nielsen et al., 2021). Global effects on miRNAs in *C. elegans* were shown in a study using developmental exposure to 10 μM MeHg from embryonic to L4 stage. This exposure led to downregulation of *miR-37–3p*, *miR-41–5p*, *miR-70–3p*, and *miR-75–3p*, concomitant with upregulation of lipocalin related (LPR) genes (involved in excretory duct cell development) and downregulation of Activated in Blocked Unfolded protein response (ABU) genes (which form part of the ER stress pathway) (Rudgalvyte et al., 2013). However, the differentially expressed genes were not predicted to be regulated by *miR-37–3p*, *miR-41–5p*, *miR-70–3p*, or *miR-75–3p*, thus the functional implications of the downregulation of these miRNAs are unclear.

5.2. Epidemiological studies

Only a few epidemiological studies have so far analysed miRNA changes in relation to MeHg. Li et al. (2015) used 43 placentas to evaluate associations between total mercury and expression of 112 miRNAs considered to be expressed in the placenta. When dichotomizing total Hg by the median (0.8 pg/g), multiple *let-7* family members were downregulated in the above the median Hg placenta group. Further, Hg above the median was associated with reduced expression of *miR-151–5p*, *miR-10a*, *miR-193b*, *miR-1975*, *miR-423–5p*, *miR-520d-3p*, *miR-96*, *miR-526aC*, *miR-518d-5pC*, and *miR-520c-5p*.

Sanders et al. (2015) analysed total Hg in toenails (biomarker of long-term MeHg exposure) and miRNA in cervical swabs of pregnant women ($n = 40$). Seventeen out of 74 miRNAs were negatively associated with toenail Hg levels: *let-7a* and *let-7b*, and miRNAs 205, 125b, 200c, 342, 203, 24, 22, 23b, 375, 23a, 210, 200b, 99a, 21, and 193b. miRNAs associated with toenail Hg were predicted to have 8446 downstream gene targets. Still, the study did not directly measure the downstream changes on RNA expression and protein translation and future characterization is needed in order to understand how cells respond to MeHg-related miRNA changes. This study and the previous study were pioneering studies evaluating the associations between MeHg and miRNA and indicated that MeHg exposure may alter miRNA expression. Still, they both contained small number of samples and limited dose-response analysis.

Human milk contains extracellular vesicles that can transport miRNAs from mother to infant. In a Faroese birth cohort, maternal Hg levels in hair during pregnancy were compared with the presence of extravesicular miRNAs in 333 milk samples collected between 2 and 74 days postpartum (Kupsco et al., 2022). No associations between Hg and expression of individual miRNAs were found after controlling for multiple comparisons. However, Hg was nominally associated with 13 miRNAs in individual regression models. Exploration of pathway

analyses suggested that miRNAs (*miR-200b-3p*, *miR-664a-3p*, *miR-6738–5p*, *miR-429*, *miR-1236–5p*, *miR-4464*, and *miR-30b-5p*) are involved in pathways of relevance for the nervous system, e.g. axon guidance and mTOR signalling. Further, studies are needed to follow-up whether these miRNA may mediate MeHg neurotoxicity. A limitation with the study was that it does not show all 13 miRNAs, but only 7, associated with Hg, neither are effect estimates provided.

Longo et al. (2022) measured the serum concentration of Hg, which reflects both inorganic Hg and MeHg, during the last trimester of pregnancy and examined its association with serum *miR-30b*, *miR-223*, and *Let-7a* expression in 68 pregnant women in southern Italy. Lower *miR-30b* expression was associated with higher Hg concentrations, both in individual analysis and when combined with organic contaminants, whereas no association was found for the other miRNAs. A strength of the study is that it, in parallel to individual associations between MeHg and miRNAs, evaluated associations for mixture of MeHg and organic contaminants.

6. Discussion

Experimental studies in cell and animal models have shown that MeHg exposure affects DNA methylation. However, the results are not consistent. Some studies show increased global methylation, while others show decreased or no effect on global methylation. Several studies show locus-specific methylation changes; however, target gene (s) vary and no common markers have yet been identified. The variability in results suggests possible inter-taxa differences in epigenetic responses to MeHg; however, animals were exposed to MeHg through different routes (dietary, egg injection), for different periods of time, and at different life stages (embryonic, juvenile, adult). Experimental studies addressing the relationship between MeHg-induced epigenetic changes and neurodevelopmental outcomes were very few, and further studies are needed.

Epidemiological studies provide some evidence that MeHg can alter DNA methylation and that prenatal exposure can lead to DNA methylation changes that persistent at least into early childhood (Cardenas et al., 2017b; Cediel Ulloa et al., 2021; Lozano et al., 2022). However, no overlapping genes were identified across EWAS studies, which showed great differences in the identity and number of CpGs identified and associations with genes with unknown relationships with MeHg. There has been limited validation of EWAS signals (Bakulski et al., 2015; Cediel Ulloa et al., 2021). The candidate gene *NRC31* showed hypermethylation with increasing prenatal MeHg exposure in two studies (Appleton et al., 2017; Cediel Ulloa et al., 2021). *NRC31* is of interest for MeHg toxicity as it is a critical gene for disease development in the nervous system; however, this gene has not been identified by EWAS studies. In addition, some studies relied on small sample sizes resulting in limited statistical power, particularly when interrogating a large number of CpG sites. Differences between the populations studied (e.g., age, sex, genetic background, nutritional status), the type of tissue examined (e.g., whole blood, cord blood), levels of MeHg, and differential residual confounding might explain the limited replication among epigenetics studies of MeHg. Nevertheless, the majority of studies were performed in U.S. study groups, which should reduce variation. Several reports suggest that *in utero* exposure can alter DNA methylation, potentially altering fetal developmental programming, which may result in a higher risk of disease later in life. Only two studies associated MeHg-induced methylation changes to neurodevelopmental outcomes (Cardenas et al., 2017b; Maccani et al., 2015). Based on the limited existing evidence, it is therefore difficult to comprehend how important the reported associations are and further studies on the functional effects of MeHg-related DNA methylation changes and their magnitude are warranted. No studies have addressed concurrent exposure to MeHg and epigenetic effects.

There is a growing body of evidence indicating that metals can induce epigenetic alterations in a sex-specific manner (Broberg et al., 2014; Cediel Ulloa et al., 2021; Kippler et al., 2013). These alterations, if functional, may account for differential susceptibility to MeHg-induced neurotoxicity in boys and girls, a feature observed in some cohorts exposed to MeHg (Bauer et al., 2020). However, none of the animal or experimental studies reviewed here evaluated sex differences in the epigenetic effects caused by MeHg. Among the epidemiological studies, we identified only a few where sex differences were evaluated. Higher levels of MeHg in maternal hair were associated with higher DNA methylation of *NR3C1* and *BDNF* in boys but not in girls (Cediel Ulloa et al., 2021). Higher MeHg was also associated with greater methylation of *HDHD1*, but only in boys (Nishizawa-Jotaki et al., 2021).

MeHg-induced DNA methylation changes have been detected in critical genes for disease development in the nervous system such as *BDNF* and *NR3C1* (Cediel Ulloa et al., 2021), while other studies have detected associations with genes with unknown relationships with MeHg (Lozano et al., 2022). The functional consequences of changes in methylation of these genes or their causal relationship with disease have not been established, and further experimental data are warranted.

Altered epigenetic marks can be metastable, and thus the effects of altered gene expression and disease risk have the potential to be transgenerational, i.e., altered phenotypes occurring in the second (in the case of male transmission and non-mammalian species) or third (in the case of female transmission) generation after an environmental exposure (Perez and Lehner, 2019). In a rat model, Nava-Rivera et al. (2021) recently provided multigenerational (up to F3) evidence of changes in global DNA methylation in gonadal tissue upon chronic exposure of parents to arsenic. Experimental studies in zebrafish up to F2 indicate transgenerational effects of MeHg (Carvan et al., 2017; Olsvik et al., 2014), but further evidence is needed from other animal models to clarify whether MeHg-induced epigenome alterations in the early life of an individual might affect the health of later generations.

Compared with those on DNA methylation, there are fewer experimental studies on histone modifications in relation to MeHg and, to our knowledge, no epidemiological studies. Experimental studies have shown histone modifications after both acute and chronic exposure, with several showing decreased H3 levels and modifications to H3 (Go et al., 2021; Go et al., 2018; Onishchenko et al., 2008). Most histone proteins are devoid of cysteine residues. Only H3 contains one completely conserved cysteine residue, which may be a direct binding site for MeHg. In yeast, this site is inessential (Dai et al., 2008), and mutation of this site could be used to test whether histone effects of MeHg occur via binding to this cysteine residue.

Experimental studies have revealed changes in multiple miRNAs, but with little overlap, in response to MeHg. In two epidemiological studies, downregulation of members of the miRNA *let-7* family was associated with higher MeHg exposure, whereas a third study did not find any association. The *let-7* family is one of the most highly conserved families of miRNAs in animals and is involved in several biological processes including differentiation, cell death, metabolism, and cancer (Chirshev et al., 2019). MicroRNAs may be promising new biomarkers for toxicity, but we currently have limited knowledge about miRNAs in general. The true number of human miRNAs is still not defined, and many of them have hundreds of target genes (Alles et al., 2019), which warrants further studies to identify miRNAs that specifically reflect an exposure and toxicity phenotype.

7. Conclusions

The existing experimental and epidemiological reports of epigenetic changes associated with MeHg exposure provides some evidence that MeHg may cause epigenetic changes. If so, they may be persistent and possibly linked to alterations in neurodevelopment. The literature is, however, still limited, and further experimental and epidemiological studies are needed.

CRediT authorship contribution statement

Karin Broberg: Conceptualization, Supervision, **Karin Broberg and Andrea Cediel Ulloa**: Writing- Original draft preparation. **Sabrina Lindner**: Investigation. **Karin Broberg and Joelle Ruegg**: Validation, **All authors:** Writing- Reviewing and Editing,

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2023.05.004.

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