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Global Mercury Partnership

ECOGENETICS OF MERCURY: FROM GENETIC POLYMORPHISMS AND EPIGENETICS TO RISK ASSESSMENT AND DECISION-MAKING

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Abstract: The risk assessment of mercury (Hg), in both humans and wildlife, is made challenging by great variability in exposure and health effects. Although disease risk arises following complex interactions between genetic ("nature") and environmental ("nurture") factors, most Hg studies thus far have focused solely on environmental factors. In recent years, ecogenetic-based studies have emerged and have started to document genetic and epigenetic factors that may indeed influence the toxicokinetics or toxicodynamics of Hg. The present study reviews these studies and discusses their utility in terms of Hg risk assessment, management, and policy and offers perspectives on fruitful areas for future research. In brief, epidemiological studies on populations exposed to inorganic Hg (e.g., dentists and miners) or methylmercury (e.g., fish consumers) are showing that polymorphisms in a number of environmentally responsive genes can explain variations in Hg biomarker values and health outcomes. Studies on mammals (wildlife, humans, rodents) are showing Hg exposures to be related to epigenetic marks such as DNA methylation. Such findings are beginning to increase understanding of the mechanisms of action of Hg, and in doing so they may help identify candidate biomarkers and pinpoint susceptible groups or life stages. Furthermore, they may help refine uncertainty factors and thus lead to more accurate risk assessments and improved decision-making. *Environ Toxicol Chem* 2014;33:1248–1258. © 2013 SETAC

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INTRODUCTION

Ecogenetics evolved from the field of pharmacogenetics and has been defined by Costa and Eaton as the "study of critical genetic determinants that dictate susceptibility to environmentally influenced adverse health effects" [1]. Contemporary ecogenetic thinking emerged in the middle to late 20th century with seminal contributions by Motulsky [2], Omenn and Motulsky [3], and Grandjean [4], reasoning that individual susceptibilities have an ecogenetic basis and that consideration of ecogenetics can have profound implications for science and policy. Only in recent years, however, has this field grown substantially. This is due largely to rapid technological advancements in the laboratory, a deeper understanding of molecular and functional genetics, and the realization that epigenetic processes serve as an interface between an organism's rapidly changing environment and its relatively fixed genome. In both ecotoxicology [5] and human health [1,6], it is now widely accepted that disease risk and progression arise from a complex interplay between genetic ("nature") and environmental ("nurture") factors (Figure 1).

Mercury (Hg) is an environmental contaminant of global concern whose risk assessment and management can benefit from an ecogenetic understanding. Reports published by expert panels and leading scientists have concluded that real-world exposure to Hg may be associated with a range of subclinical and adverse health outcomes in humans and wildlife [7–10]. These reports acknowledge that tremendous interindividual and interspecies variation exists in exposure and hazard and that complex gene–environment interactions may underlie such variation but as yet remain unresolved. With no resolution to such variability, decision-making is hampered and uncertain [11,12]. Risk assessments attempt to account for variability by utilizing default uncertainty factors. Uncertainty factors increase the margin of safety in an effort to protect sensitive subgroups, but in doing so they may still prove to be insufficient or perhaps even overprotective. As we embark on next-generation risk assessment [12], we must harness emerging ecogenetic approaches to help increase understanding of true biological variation across and within individuals and species so that uncertainty factors are refined and risk assessments improved.

To identify susceptible subgroups, ecogenetic thinking calls for attention to both environmental and genetic determinants of exposure and hazard. In ecotoxicology and human health, however, most Hg studies are focused solely on resolving environmental or exposure-related factors (e.g., what are Hg levels in fish and consumers?). Although this remains a requisite step in Hg risk assessment, it has limited use alone in predicting risk. Mercury science is approaching a point at which ecogenetic methods may now be used to better identify susceptible groups and perhaps minimize the reliance on uncertainty factors. In recent years, increasing numbers of studies have started to document genetic and epigenetic factors that may influence the toxicokinetics or toxicodynamics of Hg. For example, a study on monozygotic and dizygotic twins revealed Hg biomarker variance stems from both additive genetic effects and unshared environmental effects, with the genetic component explaining an estimated 30% of variance in Hg concentrations [13]. Epidemiological studies on populations exposed to inorganic Hg (e.g., dentists and miners) or methylmercury (MeHg; e.g., fish-consuming populations) are showing that polymorphisms in a number of environmentally

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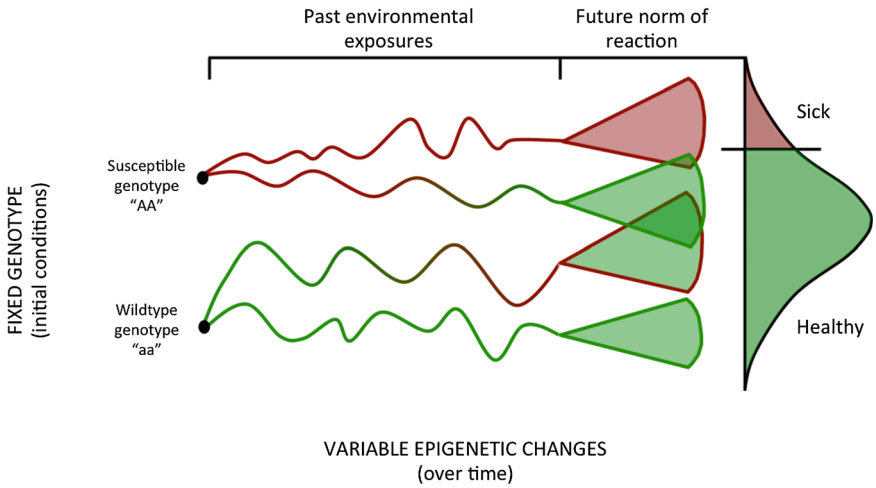

Figure 1. Ecogenetic interactions. In this hypothetical example, at conception, organisms start with a fixed genotype that renders them "healthy" (aa) or "susceptible" (AA). Over a variable time continuum (*x* axis, moving from left to right), exposure to environmental stressors can vary and influence the likelihood of an organism becoming healthy (green) or sick (red). For example, 2 genetically identical individuals with the same "susceptibility" (AA) genotype can end up with differing health outcomes based on the environment in which they developed and resided. Likewise, epigenetic changes induced by environmental exposures at sensitive time points may propagate throughout the life course and influence health outcomes. Adapted with permission from Kardia [106]. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

responsive genes can explain variations in Hg biomarker values and health outcomes (Tables 1 and 2). Studies on mammalian wildlife and humans are showing MeHg exposures to be related to epigenetic markers (Table 3). These findings, and many others, are beginning to increase our understanding of the mechanisms of action of Hg and potentially identify candidate biomarkers of susceptibility, which may lead to more accurate risk assessments and improved decision-making. The present study reviews these studies and discusses their utility in terms of Hg risk assessment, management, and policy and offers our perspectives on fruitful areas for future research.

GENETIC FACTORS AND POLYMORPHISMS

Certain genetic pathways (e.g., xenobiotic metabolism, DNA repair, cell cycle) have evolved to protect the body from environmental toxicants and stressors. The genes in these pathways, referred to as "environmentally responsive genes," have polymorphic variants that help organisms better cope with changing environments. In humans, approximately 0.1% of DNA (3 million base pairs) is variable between individuals, and some of these polymorphic loci may render individuals more sensitive or more resistant to environmental contaminants. The

Table 1. Significant associations between genetic polymorphisms and mercury biomarker levels in epidemiological studies

		Methylmercury biomarkers		Inorganic Hg biomarkers	
System	Genes	Alleles associated with higher biomarker levels	Alleles associated with lower biomarker levels	Alleles associated with higher biomarker levels	Alleles associated with lower biomarker levels
Glutathione pathway	*GCLC, GCLM, GSS, GSR, GGT1, GSTA1, GSTM1, GSTM3, GSTP1, GSTT1*	*GCLC* rs17883901 T [16,26]; *GSTP1* rs1138272 T [16,26]; *GSTP1* rs1695 G [16]; *GCLM* rs41303970 T [28]; *GSTM1* and *GSTT1* deletions [16,30]; *GSS* rs3761144 G [29]	*GSTP1* rs1695 G and rs1138272 T [28,29]; *GSTA1* rs3957396 A [16]	*GCLM* rs41303970 T [27,31] [a]	*GSTT1* deletion [29]; *GCLM* rs41303970 T [31] [a]
Metallothionein	*MT1A, MT1E, MT1G, MT1M, MT2A, MT4, MTF1*	*MT1M* rs9936741 C [32]; *MT1A* rs11640851 C [16]; *MT4* rs11643815 A [16]	*MT1A* rs8052394 G [32]	*MT2A* rs10636 C [32]	*MT1M* rs2270836 A [32]
Selenoprotein	*SEPP1, GPX1, GPX4*	None	*SEPP1* rs7579 T [29]	*SEPP1* rs7579 T [29]	None
Xenobiotic transport	*MRP1, MRP2, LAT1, LAT2, OAT1, OAT3, MDR1, SLC3A2*	Not studied	Not studied	*MRP2* rs1885301 A and rs717620 A; *LAT1* rs33916661 G [41]	*MRP2* rs2273697 A; *OAT1* rs4149170 G; *OAT3* rs4149182 C [41]

[a]T allele was associated with higher blood Hg among gold miners, merchants, and a reference population with varying inorganic Hg exposures. Blood Hg in this cohort is likely a better indicator of inorganic Hg because of very high inorganic Hg exposures. T allele was also associated with faster Hg elimination following burning of Hg-gold amalgam as observed in urine.

rs = reference SNP number; *GCLC* = glutamate-cysteine ligase catalytic subunit; *GCLM* = glutamate-cysteine ligase modifier subunit; *GSS* = glutathione synthetase; *GSR* = glutathione reductase; *GGT1* = γ-glutamyltransferase 1; *GSTA1* = glutathione S-transferase alpha 1; *GSTM1* = glutathione S-transferase mu 1; *GSTM3* = glutathione S-transferase mu 3; *GSTP1* = glutathione S-transferase pi 1; *GSTT1* = glutathione S-transferase theta 1; *MT1A* = metallothionein 1A; *MT1E* = metallothionein 1E; *MT1G* = metallothionein 1G; *MT1M* = metallothionein 1M; *MT2A* = metallothionein 2A; *MT4* = metallothionein 4; *MTF1* = metal-regulatory transcription factor 1; *SEPP1* = selenoprotein P, plasma 1; *GPX1* = glutathione peroxidase 1; *GPX4* = glutathione peroxidase 4; *MRP1* = multidrug resistant protein 1; *MRP2* = multidrug resistant protein 2; *LAT1* = large neutral amino acid transporter 1; *LAT2* = large neutral amino acid transporter 2; *OAT1* = organic anion transporter 1; *OAT3* = organic anion transporter 3; *MDR1* = multidrug resistance; *SLC3A2* = *sol*ute carrier family 3A2.

Table 2. Genotype–mercury interactions influencing health outcomes: Hg biomarkers used, genes with polymorphisms of interest, and health parameters measured for each study along with any statistically significant genotype–Hg biomarker relationships

Type of outcome	Health outcome	Genes	Hg biomarkers	Significant polymorphism–Hg biomarker interactions on health outcome	Reference
Health biomarkers	Urinary porphyrin excretion	*CPOX, UROD*	Urine	*CPOX* Asn272His ("*CPOX*4") associated with atypical pattern of porphyrin excretion among exposed individuals	[42]
	MMP-9 and MMP-2 protein levels	*MMP9, MMP2*	Blood, plasma	*MMP9* microsatellite genotype impacts MMP-9 activity in tertile with lowest plasma Hg; blood Hg modifies relationship between *MMP2* promoter polymorphism and MMP-2 levels	[49,50]
	Nitric oxide production	*NOS3*	Blood	Genotype of *NOS3* repeat polymorphism and blood Hg impact plasma nitrite levels	[51,52]
Subclinical measures	Low birth weight	*GSTM1, GSTT1*	Blood	Increased risk with *GSTM1* and *GSTT1* deletions and higher blood Hg	[48]
	Neurobehavioral tests (adults)	*BDNF, CPOX, SLC6A4* linked region (5-HTTLPR)	Urine	Additive effect of *BDNF* Val66Met and urine Hg on tests of visuomotor and finger tapping speed; additive effect of CPOX4 and urine Hg on measures of visuomotor processing and depression; additive effect of 5-HTTLPR deletion and urine Hg on finger tapping speed and hand steadiness	[43–45]
	Neurobehavioral tests (children)	*CPOX*	Urine	Interaction between CPOX4 and urine Hg on several neurobehavioral tests in boys	[47]
	Mood, neurological symptoms	*BDNF, COMT, 5-HTTLPR*	Urine	Additive effect of *BDNF* Val66Met with urine Hg on anxiety and memory	[46,54,55]
	Peripheral nerve conduction	MTs, GSH pathway, selenoproteins	Hair, urine	None	[56]
Clinical measures	Tremor, neuromotor tests	GSH pathway	Blood, urine	None	[31]
	Myocardial infarction	GSH pathway	Erythrocyte	None	[53]

CPOX = coproporphyrinogen oxidase; *UROD* = uroporphyrinogen decarboxylase; *MMP9* = matrix metallopeptidase 9; *MMP2* = matrix metallopeptidase 2; *NOS3* = nitric oxide synthase 3; *GSTM1* = glutathione S-transferase mu 1; *GSTT1* = glutathione S-transferase theta 1; *SLC6A4* = solute carrier family 6; 5-HTTLPR = serotonin-transporter-linked polymorphic region; *BDNF* = brain-derived neurotrophic factor; *COMT* = catechol-O-methyltransferase; MT = metallothionein; GSH = glutathione.

most common type of genetic variability, single nucleotide polymorphism (SNP), occurs when 1 nucleotide is altered in the genome sequence, and the variant allele is found in at least 1% of the population. Although not all SNPs have known phenotypic effects, many have been shown to confer resistance or susceptibility to disease [14], impact drug metabolism and efficacy [15], and influence toxicokinetics and toxicity of chemicals or heavy metals. This third phenotypic effect has been well studied for Pb [16,17], As [18,19], and Be [20,21]. For Hg, as outlined below, the knowledge of gene–Hg relationships is now growing.

Gene classes important to Hg toxicokinetics

Genes involved in the toxicokinetics of Hg include those underlying glutathione (GSH) function (e.g., glutathione S-transferases [GSTs]), proteins that bind and transport Hg (e.g., selenoproteins, metallothioneins [MTs]), and xenobiotic transporters (e.g., multidrug resistance proteins [MRPs]). Polymorphisms in these environmentally responsive genes are ubiquitous across populations and thus may influence the absorption, distribution, metabolism, and elimination of Hg. Below, we review key findings relating to the impact of polymorphisms in these genes and their potential to affect Hg toxicokinetics.

Glutathione. Glutathione binds inorganic Hg and MeHg, allowing cellular efflux (e.g., via MRPs) and eventually fecal excretion [22]. Polymorphisms in GSH synthesis pathway genes (*GCLM, GCLC, GSS, GSR*) could impact the bioavailability of GSH for this process. Additionally, GSTs (*GSTP1, GSTA1, GSTM1, GSTM3, GSTT1*), a highly polymorphic family of enzymes, may catalyze the conjugation reaction or may act as transporters of Hg-GSH conjugates [22]. It has been shown that 2 nonsynonymous *GSTP1* SNPs (reference SNP number [rs]1695 and rs1138272) can alter enzyme activity and sensitivity to Hg inhibition in vitro [23]. The metabolism of Hg can be affected by variation in the expression or activity of γ-glutamyltransferase 1 (*GGT1*), which cleaves Hg-GSH conjugates prior to cellular uptake [24]. In mice with a *Ggt1* knockout, more MeHg is excreted and less inorganic Hg is accumulated in the kidneys following treatment with Hg species [25].

Table 3. Summary of studies exploring epigenetic effects of mercury

Species	Tissue/cell type	Chemical	Effect	Reference
Polar bear	Brainstem	MeHg	Reduced global DNA methylation in male bears but not in female bears	[67]
Mink	Occipital cortex	MeHg	Reduced global DNA methylation, reduced DNMT activity	[68]
Chicken	Cerebrum	MeHg	No effect on global DNA methylation or DNMT activity	[68]
Yellow perch	Telencephalon	MeHg	No effect on global DNA methylation	[68]
Earthworm	Whole	Hg	Reduced global DNA methylation	[71]
Mouse	Brain hippocampus	MeHg	Suppression of the *Bdnf* promoter via hypermethylation, increased histone H3K27 trimethylation, and decreased histone H3 acetylation	[73]
Mouse	Embryonic stem cells	Hg	Reversible alterations to heterochromatin Hypermethylation of *Rnd2* gene	[75]
Mouse	Embryonic stem cells	Hg	Reduction of total histone protein levels and H3K27 monomethylation	[74]
Rat	Liver	MeHg	Reduced *Dnmt1* and *Dnmt3b* mRNA expression, decreased CpG methylation at *Cdkn2a* promoter, and no effect on global DNA methylation or SAM abundance	[70]
Rat	Primary cultures of embryonic cortical neural stem cells	MeHg	Decreased global DNA methylation, and downregulation of *Dnmt3b* mRNA	[69]
Human	Blood	Hg	Hypermethylation of the *GSTM1/5* promoter	[76]
Human	Buccal cells	MeHg	Hypomethylation of *SEPP1* gene among males	[77]

MeHg = methylmercury; DNMT = DNA methyltransferase; SAM = S-adenosylmethionine.

Recent epidemiological studies have linked polymorphisms in GSH pathway genes to hair, blood, and urine Hg levels (Table 1) [16,26–31]. Note that hair reflects exposure to organic Hg, blood generally reflects exposure to organic Hg (though some inorganic may be present in blood), and urine generally reflects exposure to inorganic Hg (though some of this may have been derived from organic Hg that was demethylated inside the body). Collectively, 12 polymorphisms in 10 GSH pathway genes have been genotyped in various population studies. Most of the polymorphisms were associated with Hg concentrations in at least 1 study and at least 1 type of statistical analysis (e.g., association with median biomarker levels, effect modification on relationship between estimated exposure and biomarker concentration). Despite the use of populations with various ethnicities and Hg exposure levels, some gene–Hg relationships were similar across studies. For example, minor alleles of SNPs in glutamyl-cysteine ligase (rs17883901 in *GCLC*, rs41303970 in *GCLM*) were associated with increased MeHg biomarker levels [16,26,28]. In contrast, the *GCLM* rs41303970 minor allele was associated with higher blood, plasma, and urine Hg levels in Ecuadorian gold miners [27,31]. Not all gene–Hg relationships were consistent across studies. For example, Custodio et al. [26] found higher erythrocyte Hg levels in individuals with the minor allele of *GSTP1* rs1138272 compared with polyunsaturated fatty acid levels, an indicator of fish consumption, whereas Engström et al. [28] observed the opposite relationship. Similarly to the latter study, lower hair Hg levels were associated with minor alleles of *GSTP1* rs1138272 and rs1695 in a cohort of dental professionals with fish consumption patterns similar to those of the US general population [29].

Metallothionein. Metallothioneins bind metals, including Hg, and in doing so influence heavy metal distribution and protect against toxicity. Two epidemiological studies have explored the impact of MT and MT transcription factor (MTF) SNPs on Hg biomarkers [16,32]. In a cohort of dental professionals, several significant interactions between exposure source and genotype were observed in models of hair Hg (*MT1M* rs9936741, *MT1A* rs8052394) and urine Hg (*MT1M* rs2270837, *MT2A* rs10636) [32]. Gundacker et al. [16] additionally found 2 SNPs (*MT1A* rs11640851, *MT4* rs11643815) to be associated with higher hair Hg.

Selenoprotein. Selenoproteins buffer against Hg-induced oxidative stress via Hg binding [33,34]. With up to 10 selenocysteine residues per protein, selenoprotein P1 (SEPP1) is particularly equipped for this task [34,35]. The genotype of *SEPP1* influences isoform prevalence and gene expression, and variation in these parameters may influence Hg sequestration or tissue distribution [36–38]. In a cohort of dental professionals, a regulatory-region *SEPP1* SNP (rs7579 in the 3'-untranslated region [3'UTR]) was differentially associated with hair Hg and urine Hg levels [29]. A significant interaction between fish intake and genotype led to lower hair Hg, whereas genotype and dental amalgam exposure interacted to increase urine Hg among more highly exposed individuals. Both Hg exposure [33] and polymorphisms in the encoding genes [39,40] influence expression and activity levels of glutathione peroxidases (GPX), antioxidant proteins typically containing 1 selenocysteine residue. Despite a plausible gene–Hg interaction, only 2 GPX polymorphisms have been studied to date, and no significant relationship was observed [29].

Xenobiotic transporter. Xenobiotic transporters, including the MRP and organic anion transporter (OAT) families, regulate cellular influx and efflux of GSH-Hg or cysteine-Hg conjugates. In a study of occupationally exposed artisanal gold miners from 4 countries in Africa and Asia, 18 SNPs in 8 transporter genes were studied; of these, 3 SNPs (*MRP2* rs1885301, rs717620; *LAT1* rs33916661) were linked to higher urine Hg levels in at least 1 population, and 3 were associated with lower urine Hg (*MRP2* rs2273697, *OAT1* rs4149170, *OAT3* rs4149182) [41].

Evidence of genetic factors impacting Hg toxicodynamics

Beyond the influence of genetic polymorphisms on Hg toxicokinetics and biomarker values, growing evidence links SNPs and other types of genetic polymorphisms to variability in Hg-associated adverse health outcomes (Table 2).

A large portion of studies to date involving the interaction of polymorphisms and Hg exposure on health outcomes stem from a single research team that has studied a cohort of male dentists and female dental assistants with occupational elemental (inorganic) Hg exposure (Table 2). In these studies, genetic polymorphisms modified Hg-health outcome relationships or added to the risk for the following health effects: 1) atypical urinary porphyrin excretion—indicative of Hg-induced alterations to the renal

heme biosynthesis pathway—and a nonsynonymous SNP in *CPOX* (rs1131857; called CPOX4) [42]; 2) performance on several neurobehavioral tests including hand steadiness and finger tapping with a nonsynonymous SNP in *BDNF* (rs6265) [43]; 3) the Symbol Digit Rate test (visuomotor processing) and Beck's depression factor with CPOX4 [44]; 4) a repeat deletion in the promoter of *SLC6A4* (called the 5-HTTLPR short genotype) with finger tap and hand steadiness tests [45]); and 5) *BDNF* rs6265 with indicators of anxiety and memory [46]. Some of the additive effects between Hg exposure and genotype were gender and occupation specific. Interactions of CPOX4 were also found among male children (ages 8–18) with similar urine Hg levels to the dental cohort, though these relationships were generally not observed among female children [47]. Collectively, these studies highlight the importance of polymorphisms directly related to specific health outcomes in conferring susceptibility to Hg's toxic effects. The *BDNF* gene (rs6265) appears to be a key polymorphism associated with several neurobehavioral effects, and other such polymorphisms may remain unidentified.

Besides inorganic Hg, interactions have been observed between blood Hg concentrations, indicative primarily of MeHg exposure, and polymorphisms influencing adverse health outcomes or health biomarkers. The offspring of mothers with *GSTM1* and *GSTT1* deletions and higher blood Hg levels had increased risk for low birth weight [48]. Genotype of a repeat polymorphism in matrix metalloproteinase, *MMP-9*, affected MMP-9 protein levels among fish-consuming Brazilians with the lowest plasma Hg levels, and higher MMP-9 levels were associated with multiple adverse cardiovascular effects [49]. In the same cohort, an *MMP2* promoter polymorphism (rs243865) affected MMP-2 levels when blood Hg was taken into accounted [50]. Genotype of a tandem repeat polymorphism in *NOS3* and blood Hg levels are linked to decreased nitric oxide production, another biomarker of cardiovascular health, among highly exposed fish-eaters in Brazil [51]. However, 2 *NOS3* SNPs (rs2070744, rs1799983) did not impact plasma nitrite levels following fish consumption [52].

Not all studies exploring gene–Hg interactions showed significant links with the assessed health outcomes [31,52–56], emphasizing the importance of polymorphism selection, Hg exposure level, and health outcome measure. Most studies with significant associations genotyped variants in pathways related to the health outcome of interest. Polymorphisms involved in Hg toxicokinetics may be less likely to impact target organ toxicity, as noted from the lack of significant interactions in several studies of health outcomes and polymorphisms primarily in the GSH pathway [31,53,56].

EPIGENETIC FACTORS

Epigenetics is emerging as important to the toxicity of many classes of environmental contaminants [57,58], including some metals [59,60]. "Epigenetics" refers to factors affecting gene expression that are heritable but occur outside of direct changes to the DNA sequence. Examples of epigenetic endpoints are histone modification, DNA methylation, and RNA interference. These endpoints are affected by environmental stimuli such as chemical exposure, stress, parental behavior, and nutritional deficits. Epigenetic marks left by such stimuli can persist in tissues as cells divide in the absence of the initial stressor. This implies that exposures to chemicals at an early life stage can lead to adverse health outcomes later in life, or even in subsequent generations. These characteristics have been demonstrated in several models [57] and have important implications for environmental toxicology [5].

The epigenetic effects of Hg are a relatively new field of inquiry, especially compared with other contaminants such as As, Pb, air pollution, and endocrine disruptors [58]. The epigenome is established during development and is thought to be sensitive to environmental influences during this time. Likewise, MeHg has been proven to be a developmental neurotoxin in a number of organisms [61], including humans and wildlife [62]. Another point of epigenetic consideration is that MeHg is known to have a long latency period between exposure and adverse health outcomes. For example, at Minamata Bay in Japan, impaired cognition and dysfunctions in mood and behavior were found in adults of varying ages who did not exhibit any clinical effects following developmental, early-life exposures [63]. Longitudinal birth cohort studies from the Faroe Islands have documented that developmental exposures (as determined by cord blood Hg) can be related to later-life adverse neurological outcomes in children aged 7 and 14 yr [64]. A latency period of several decades is suggestive of an epigenetic mode of action, although this has yet to be demonstrated.

Global DNA methylation

DNA methylation is a biochemical process that results in the addition of a methyl group to a nucleotide [65,66]. A growing body of research suggests that environmentally relevant MeHg concentrations can disrupt DNA methylation in mammals (Table 3). This effect has been observed in both laboratory animals and field studies. Pilsner et al. [67] report an inverse relationship between brainstem Hg levels and global DNA methylation in Greenlandic male polar bears (*Ursus maritimus*). Similarly, DNA methylation was reduced in the occipital cortex of juvenile male mink (*Neovison vison*) raised on a diet of 1 ppm MeHg for 2 mo, although DNA methylation was unchanged in animals fed 2 ppm [68]. In these same mink, activity of DNA methyltransferase (DNMT), the enzyme that establishes and maintains patterns of DNA methylation in the genome, was also reduced. In primary cultures of rat cortical embryonic neural stem cells, subtoxic concentrations of MeHg were associated with reduced neural stem cell proliferation, decreased global DNA methylation, and a slight reduction in *Dnmt3b* mRNA [69]. These effects persisted in daughter cells that were never directly exposed to MeHg-contaminated media. Several other studies support a link between MeHg exposure and reduced DNMT expression. Desaulniers et al. [70] report an MeHg-associated reduction in *Dnmt1* and *Dnmt3b* mRNA in the livers of female rats exposed to MeHg during the perinatal period. They did not, however, observe any effect on global DNA methylation or levels of the important methyl donor S-adenosylmethionine (SAM).

It is not clear whether the MeHg-associated depression in global DNA methylation observed in mammals extends to other classes of animals. In telencephalons of MeHg-fed yellow perch (*Perca flavescens*), there was no change in DNA methylation [68]. In cerebra of chickens (*Gallus gallus domesticus*) exposed in ovo, an MeHg-dependent decrease in global DNA methylation was observed, but this trend was not statistically significant [68]. In these same chickens, there were no MeHg-dependent changes in the heart or liver. In earthworms, global DNA methylation was reduced in relation to elevated levels of Hg, As, Se, and Sb. This relationship appeared to be driven mainly by As, a metal with well-described epigenetic effects [58–60], but Hg was also a contributing element [71].

We, as well as others, have suggested that global DNA methylation could be useful as a biomarker for exposure to Hg and other chemical agents [5,58]. It is important to note that, although global DNA methylation may be a general indicator for epigenetic stress, we are not aware of any studies that link Hg-associated global hypomethylation to specific health outcomes. In general, abnormal patterns of methylation have been associated with cancer and neurological, developmental, immunological, and age-related disorders [72]. More work is required to identify which areas of the genome are affected by Hg-associated hypomethylation and what, if any, adverse health outcomes are associated with reduced methylation status, particularly in the developing embryo.

Gene-specific effects

In contrast to interpreting studies concerning global changes in DNA methylation, gene-specific alterations in epigenetic status may be more easily interpreted in relation to health outcomes, In an animal study, Onishchenko et al. [73] linked MeHg-associated epigenetic repression of brain-derived neurotrophic factor (BDNF) to depressive-like symptoms in young male mice. Exposure to MeHg occurred through maternal dietary supplementation from gestational day 7 until 7 d after birth. Along with depressive-like symptoms, MeHg-exposed male offspring exhibited reduced *Bdnf* mRNA in the dentate gyrus. This was attributed to a repressive chromatin state at the *Bdnf* promotor. Onishchenko et al. observed DNA hypermethylation along with increased histone H3K27 trimethylation at *Bdnf* promoter IV and decreased histone H3 acetylation. These effects persisted in 14-mo-old mice without any further exposure to MeHg. An in vitro study using mouse embryonic stem cells found that acute exposure to Hg^{2+} reduces total histone protein levels and H3K27 monomethylation [74]. The authors speculate that this may indicate transcriptional repression. Arai et al. [75] reported that the *Rnd2* gene is hypermethylated in Hg-treated mouse embryonic stem cells. In contrast, Desaulniers et al. [70] found that the promoter of the tumor suppressor gene $p16^{INK4a}$ was hypomethylated in MeHg-exposed female rats.

Two human epidemiology studies have reported MeHg-associated DNA methylation of specific genes. In a pilot study of 58 women, hypermethylation of the *GSTM1* promoter in whole blood was found in those with blood Hg values above 2.9 μg/L [76]. In a study of 131 dental professionals, biomarkers of MeHg exposure (hair) and elemental Hg exposure (urine) were related to DNA methylation at global repetitive elements (long interspersed elements [LINE-1]) and promoter regions of genes related to epigenetic processes (*DNMT1*) and Hg interactions (selenoproteins *SEPW1* and *SEPP1*) [77]. After a series of multivariable linear regressions, the only statistically significant association was hypomethylation of *SEPP1* with hair Hg levels among males.

Mechanisms

The mechanisms by which Hg affects epigenetic endpoints have not been resolved. In general, Hg has a strong affinity for protein thiol and selenol groups, and this is thought to be the root cause of toxicity [78]. Accordingly, the direct and indirect targets for Hg are numerous and may include targets involved in maintaining cellular homeostasis, transducing signals, and ensuring redox states [79]. Here we focus on possible mechanisms by which Hg might affect DNA methylation. We recognize that other epigenetic mechanisms may be affected by Hg (e.g., histone modification or RNA interference), although DNA methylation is the most well studied epigenetic endpoint to date and is the focus below (Figure 2).

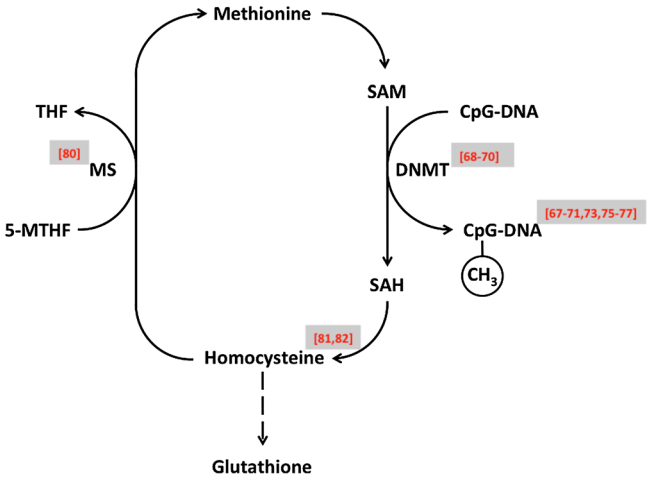

Figure 2. Mercury-associated effects on DNA methylation and the methionine cycle. Superscript numbers within shaded boxes refer to studies that show Hg exposure being associated with that particular compound. SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine; DNMT = DNA methyltransferase; MS = methionine synthase; 5-MTHF = 5-methyltetrahydrofolate; THF = tetrahydrofolate. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

DNA methylation is catalyzed by the enzyme DNMT, which facilitates the transfer of a methyl group from the universal methyl donor, SAM, to DNA (Figure 2). The covalent addition of a methyl group to DNA (usually to cytosine residues) is generally associated with the transcriptional silencing of gene expression. There is some evidence that MeHg exposure is associated with reduced expression or biochemical activity of DNMT [68–70], but it is not known whether Hg can directly bind to and inhibit the function of DNMT or whether Hg-associated changes in DNMT function are secondary effects. For example, reactive products arising from Hg-induced oxidative stress themselves might affect epigenetic endpoints. DNMT does not operate in isolation, and thus consideration also should be given to potential impacts of Hg on complementary enzymes such as EZH2 (or lysine N-methyltransferase), which helps recruit DNMTs to particular sites on DNA and also methylates histones.

Another potential target for Hg is SAM. S-adenosylmethionine is regenerated from S-adenosylhomocysteine (SAH) via the methionine cycle. Onishchenko et al. [60] suggest that the ratio of SAM to SAH, sometimes referred to as the methylation capacity, may be key to understanding how contaminants affect DNA methylation. A low ratio of SAM to SAH may result in a deficit of methyl groups for DNA methylation.

Other aspects of the methionine cycle are also important to both DNA methylation and Hg toxicokinetics. Methionine is an essential amino acid that serves as a penultimate methyl donor given that L-methionine is converted into SAM by the enzyme SAM synthetase. It is interesting to note that MeHg can form conjugates with L-cysteine and the resulting MeHg-L-cysteine complex is structurally similar to methionine. In fact, it has been documented that MeHg-L-cysteine complex can act as a molecular mimic, substituting for methionine in some cellular processes [78], including, potentially, the methionine cycle. Another potential target for Hg is methionine synthase, the enzyme that converts homocysteine to methionine. It has been speculated that methionine synthase could be involved with MeHg-associated changes to DNA methylation status [69];

methionine synthase is inhibited by inorganic and ethylated Hg [80], and the same may be true for MeHg. Inorganic Hg and MeHg may also bind with homocysteine, and these Hg-homocysteine conjugates are biologically transportable [81]. An epidemiological analysis of US National Health and Nutrition Examination Survey (NHANES) data suggest that blood Hg levels may be inversely associated with plasma homocysteine levels in younger males [82]. Collectively, these studies show that Hg may affect the methionine cycle in a number of ways, and this may subsequently impact the availability of SAM for DNA methylation.

APPLICATIONS OF ECOGENETICS TO RISK ASSESSMENT

Identifying individuals and organisms susceptible to a contaminant such as Hg remains at the forefront of ecological and human health risk assessment. Mercury is considered a priority pollutant by agencies worldwide (e.g., US Environmental Protection Agency, US Agency for Toxic Substances and Disease Registry, Health Canada, World Health Organization [WHO]), and under the 2013 Minamata Convention it is proposed to be phased out of many intentional-use products. Nonetheless, as a persistent chemical, Hg remains globally dispersed, and levels are projected to increase further in many areas [83]. Mercury exposures to humans and wildlife will be commonplace for the foreseeable future with diet (e.g., fish, rice) and proximity to point sources or certain products (e.g., small-scale gold mining, dental amalgams) remaining prominent. For a number of ethical, legal, and social reasons, it remains judicious for us to ensure that all individuals and organisms—the average and the most sensitive—are identified and protected. In this section, we discuss how the ecogenetic research reviewed in the present study may be used to help improve the risk assessment of Hg and ultimately lead to better decision-making.

Uncertainty factors

Risk assessors in both ecotoxicology and human health apply uniform uncertainty factors to account for inherent variability [84,85]. In the early 2000s, the US National Academy of Sciences/National Research Council (NAS/NRC) and the Joint Expert Committee on Food Additives and Contaminants (JECFA) under the Food and Agriculture Organization and the WHO calculated a lower limit benchmark dose for MeHg (see Mergler et al. [9] for discussion). In deriving this dose, uncertainty factors were applied to account for unquantifiable matters such as interindividual variability and adequacy of findings. The NAS/NRC applied an uncertainty factor of 10 to account for uncertainty divided equally between toxicokinetics (3.2×) and toxicodynamics (3.2×). The JECFA report applied an uncertainty factor of 6.4 to account for variability in toxicokinetics (3.2×) and toxicodynamics (2.0×). Though necessary, such uncertainty factors can have a profound influence on managing risk. For example, the NAS/NRC used a cord blood Hg value of 58 μg/L as their benchmark dose lower limit. After the application of a 10-fold uncertainty factor, the benchmark value dropped to 5.8 μg/L. Although 58 μg/L is a value few would exhibit, 5.7% of women of child-bearing age (16–49 yr) in the United States have blood Hg levels that exceed 5.8 μg/L, with percentages being higher in certain subpopulations (e.g., 16.6% of those self-identified as Asian, Pacific Islander, Native American, or multiracial [86]), according to the NHANES 1999–2002 survey. With a great number of individuals potentially exposed to MeHg at levels deemed harmful, fish consumption advisories have been issued by many jurisdictions worldwide. Advisories may result in pregnant women reducing fish consumption, and although such reductions would also decrease MeHg exposure, they would also decrease exposure to key nutrients in fish [87]. Reduced intake of such nutrients may result in adverse health outcomes.

Can an ecogenetic approach refine uncertainty factors and thus better identify sensitive populations? The ultimate goal would be to describe the genetic components underlying MeHg variability so that we can reduce uncertainty and utilize individualistic benchmark values that are protective yet still allow the benefits of fish consumption. Here we provide a simplistic, yet plausible, scenario based on one of our studies. We calculated estimated Hg intake (micrograms Hg per kilogram body weight per day) based on self-reported fish consumption (e.g., portion size, consumption frequency for 28 fish species) and average species-specific Hg levels [29]. As expected, estimated Hg intake predicted hair Hg levels among 469 individuals. However, inclusion of *SEPP1* rs7579 genotype in the model, a regulatory region SNP, drastically changed predicted hair Hg concentrations depending on genotypic subgroup. For nonfish consumers, regardless of genotype, the model predicted similar hair Hg levels. However, for fish consumers, genotype was an important contributor. Hair Hg predictions for frequent fish consumers (equivalent of 6 cans of tuna per week) varied 8-fold depending on genotype: 6.16 μg/g among individuals with the CC genotype compared with 2.14 μg/g (CT) and 0.74 μg/g (TT).

Exposure biomarkers

A major limitation in the risk assessment of Hg is the tremendous interindividual variability observed in exposure biomarkers. For example, in humans, a hair to blood ratio of 250 is used in risk assessment, but this value can vary widely among populations (mean, 140–370) and individuals (maximum ratio >600) [88–90]. Calculated half-lives of MeHg range from 33 d to 120 d in hair, 99 d to 120 d in blood cells, and 47 d to 130 d in plasma [91]. Variable inorganic Hg retention has also been observed in humans [92] and mice [93], the latter of which is strain and gender dependent. Similarly for wildlife, Hg levels in samples collected noninvasively, such as fur and feathers, can vary widely compared with internal doses measured in blood or organs [94,95]. When such data are utilized in validated exposure assessment models, great discrepancy can exist between the modeled relationships and the measured biomarker values. For example, in a reanalysis of fish consumption surveys and measured biomarker values from a number of human groups worldwide, Canuel et al. [96] documented that the normative risk assessment of MeHg yielded results that were highly variable (68–573%) in terms of relating modeled hair Hg with measured hair Hg. A number of factors may help explain this variability (e.g., recall bias, instrument error), although, as discussed by the authors, it is also quite likely that unresolved interindividual genetic differences feature prominently. As outlined in Table 1, the first ecogenetic studies are showing that polymorphisms in genes within the glutathione, metallothionein, selenoprotein, and xenobiotic transporter families may affect Hg biomarker values; and it is hoped that, in the future, consideration of such information may help to improve the interpretation of exposure biomarkers, as discussed in the review article by Dorne et al. [97].

Interspecies differences

The intraspecies differences in Hg toxicokinetics and toxicodynamics observed in humans also exist for fish and

wildlife but have received minimal study. More attention is paid to differences across species. For example, Heinz et al. [98] injected MeHg into eggs of 26 bird species, found variable dose–response curves and calculated LC50s, and provided some discourse on sensitivity differences based on taxonomy. For marine mammals, polar bears are exposed to some of the greatest Hg levels among all organisms (liver concentrations >100 ppm dry wt), but surprisingly low levels were found in their brains (<1 ppm dry wt measured, but >20 ppm expected) [99]. Within the Mustelidae family, mink and river otters inhabit common ecosystems but seem to have different abilities to metabolize Hg in the brain. Compared with otters, mink have a lowered ability to demethylate organic Hg and accumulate Se in the brain [100]. Similar differences in demethylation and Se accumulation have also been found in common loons and bald eagles [101]. In these selected examples, the interspecies differences have been discussed nearly exclusively in consideration of factors such as natural history and physiology, although it is likely that ecogenetics also plays a role. As an example, a previous study documented that 2 amino acid changes in the avian aryl hydrocarbon receptor could help predict species sensitivity to dioxin-like compounds [102]. We wonder whether similar genetic differences may be uncovered for Hg to help improve ecological risk assessment.

Nutrient–toxicant interactions

As mentioned previously, fish are not only a major source of MeHg but also an important source of essential nutrients, such as omega-3 fatty acids, that may have positive effects on physiological systems adversely affected by MeHg. A number of nutrients or nutritional factors, such as Se, cysteine, protein, milk, and vitamin E have been shown to affect the toxicokinetics and toxicodynamics of Hg [103]. Much of this evidence has been gleaned from in vitro or animal studies, although within human epidemiology there remains continued debate concerning the protective effects of various nutrients on MeHg-associated adverse health outcomes [9]. Perhaps some of this debate stems from the fact that many nutrients, like toxicants, have a strong ecogenetic basis [104]. For example, an increase in plasma Se concentrations following a 6-wk supplementation trial varied significantly based on genotype of 2 *SEPP1* SNPs (rs7579, rs3877899), body mass index, and interaction between body mass index and genotype [36]. Within epigenetics, several studies have documented that altered exposure of animals and people to dietary methyl donors (e.g., folate, choline) is related to predicted alterations in DNA methylation [105]. Although the task of linking ecogenetic data from Hg and a number of nutrients into a single study to help tease apart risk–benefits seems daunting, advances in statistical modeling may prove useful.

Vulnerable windows and latencies

MeHg is a developmental toxicant, known to exhibit a long latency of effect. This makes it difficult to relate early-life exposures and later-life health impacts causally and thus represents a major challenge to risk assessment. Although much more research is needed, epigenetics is starting to provide mechanistic evidence to bridge the temporal gap between exposure and disease. Mercury-induced changes in DNA methylation, for example, may result in gene expression changes that are persistent and traceable. For example, in a study of neural stem cells that were exposed to MeHg, changes in DNA methylation were found in cells directly exposed but also in their daughter cells that were never exposed to MeHg [69]. In other words, altered DNA methylation may serve as a "memory" of contaminant exposure [66]. This could potentially help identify vulnerable windows of susceptibility to toxic effects.

CONCLUSIONS

Mercury is a contaminant of global concern and will remain so in the foreseeable future. The risk assessment of Hg and subsequent management decisions have been hampered by great variability in exposure and health effect, in both humans and wildlife. With MeHg exposure in humans as an example, debate continues concerning interindividual variation in adverse neurodevelopmental outcomes (e.g., differing outcomes in Faroe vs Seychelles studies), progression of cardiovascular disease (e.g., hypertension risk), and latencies between exposure and health effect, which can range from weeks to years as was observed following the Minamata Bay incident [8–10]. To date, most studies have tried to account for variability by considering, for example, pertinent biological or environmental factors such as age and gender and coexposures to other toxicants and nutrients; inclusion of such covariates has met with limited success. There is now growing evidence, as highlighted in the present study, that an ecogenetic understanding (i.e., genetic polymorphisms, epigenetic processes) may help uncover underlying mechanisms of action and better identify susceptible subpopulations. The field is in its infancy, and a number of potentially exciting avenues of research are emerging as the field moves forward. Below, we provide a few examples for both genetics and epigenetics.

In genetic studies, first, discrepancies among epidemiological studies seemingly related to differences in exposure characteristics (e.g., dose, source, Hg species, duration), population genotype frequencies, Hg biomarkers analyzed, statistical modeling, and statistical power highlight the need for improved study designs and communication among research teams. Standardizing methods across research groups for estimating Hg exposures (e.g., fish consumption surveys vs measuring fish biomarkers in blood), Hg body burden (collection of all 3 main biomarkers), and toxicity (e.g., neurobehavioral test batteries) whenever possible would improve comparability of studies and increase the weight of evidence for important genetic factors. Second, the total number of polymorphisms studied to date has been small, with the majority focused on GSH pathway genes. Hypothesis-driven, candidate gene research involving environmentally responsive genes should continue, although gene selection could be organized in a pathway-based manner (i.e., multiple genes in a given pathway investigated simultaneously). In addition, technological advances and reduced costs are now permitting the analysis of millions of genetic variants via SNP arrays. Such genome-wide association studies may help uncover key polymorphisms in genomic regions that have been unexplored or not previously considered to be important in Hg risk. Statistical modeling improvements will help move the field toward a more realistic picture of how SNPs may impact Hg risk. Third, we are not aware of genetic polymorphism studies in fish and wildlife in relation to Hg exposure, and this represents a potentially fruitful area of research, given that DNA sequencing costs are not prohibitively expensive.

In epigenetic studies, first, the total number of studies concerned with Hg is limited, so there is a great need to perform the most basic studies to increase the knowledge base. For example, research on whether Hg affects DNMT activity, methionine synthase activity, and SAM levels must be resolved at the observational, experimental, and mechanistic levels.

Global DNA methylation data are of limited use, and future work should focus on methylation of specific gene targets. Beyond DNA methylation, other epigenetic markers (e.g., histone modifications) exist that have received very little attention in terms of Hg exposure. Without such basic knowledge, interpreting the outcomes of more sophisticated research outcomes will be challenging. Second, given the promise of epigenetics as a mechanism to help explain long latencies and transgenerational effects, carefully designed longitudinal human studies and multigenerational wildlife studies will need to be conducted. Third, as with genetics, platforms now exist by which thousands of DNA methylation marks can be interrogated. Use of such epigenome-wide association technologies will reveal a greater picture of regions of the epigenome targeted by Hg and may provide mechanistic links to toxic outcomes.

Mercury is a contaminant that is being acted on by decision-makers worldwide, from local settings to the global level. As we embark on activities that will assess the efficacy of these decisions, we will need to rely on scientific advancements and next-generation risk assessments. It will be necessary to incorporate emerging ecogenetic approaches to help increase understanding of the true biological variation across and within species so that uncertainty factors are refined and risk assessments improved. The tools and basic knowledge for conducting such studies are now at our disposal. Such activities will need to be conducted with a constant reminder that disease risk and progression arise following complex interactions between genetic ("nature") and environmental ("nurture") factors over time.

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