Alteration of urinary metabolic profile in rats treated with methylmercury

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INTRODUCTION

Human exposure to methylmercury (MeHg), a widespread environmental contaminant, through consumption of contaminated fish, continues to pose a significant health concern. MeHg is a potent neurotoxicant in humans, especially in early developmental stages (Ceccatelli et al. 2010). A growing body of evidence also suggests that MeHg exposure may also lead to increased risks of adverse cardiovascular impacts in exposed populations (Rice et al. 2010). In addition, the kidney is also a known target organ of mercury accumulation and toxicity (Jin et al. 2009).

MeHg interacts with selenol and thiol groups of small molecules and enzymes involved in various metabolic pathways (Carvalho et al. 2008). A metabolomic approach was used to detect changes in urinary metabolic profile of rats treated with methylmercury (MeHg) and identify potential biomarkers of exposure and toxic effects.

METHODOLOGY

Animal treatment

Weanling male Sprague–Dawley rats were administered a semi-purified isocaloric diet containing either soy oil, seal oil, docosahexaenoic acid (DHA), fish oil or lard for 28 days.

Animals were then gavaged with either 0, 1 or 3 mg MeHg/Kg body weight (bw) per day and fed the same diet for 14 days.

An aliquot of a 24-h urine sample collected on the day of necropsy (day 14 of Me-Hg treatment) was extracted and the extract successively methoxymated and trimethylsilylated prior to analysis by GC/TOF-MS (Taylor et al. 2010).

GC-TOF-MS

Agilent 6890 gas chromatograph – Leco Pegasus IV Tof MS

Gerstel MPS2/ALEX dual rail automatic liner exchange autosampler

Cold injection onto a 30 m-long, 0,25-mm ID Rtx5Sil-MS column (10-m integrated guard column).

DATA PROCESSING AND STATISTICAL ANALYSIS

ChromaTOF v 2.32 was used for data preprocessing and further processing was performed by filtering algorithm implemented in BinBase database (Taylor et al. 2010).

Compound were identified by matching retention index (RI) and one major mass spectrum ion to compounds in FiehnLib (containing 1,200 authentic spectra and RI).

Statistical analyses were conducted using MetaboAnalyst web server on log-transformed peak intensities (Xia et al. 2009). The STATISTICA software was used for Box & Whisker plots and Cytoscape for creating metabolic network diagrams.

RESULTS

Between 300 and 350 annotated metabolites were identified in each urine sample, roughly 50% of which were unambiguously identified.

One-way ANOVA analyses revealed highly significant differences in peak intensities between MeHg treatment groups (Table 1). Examples of different diet- and treatment-related effects are shown in Figure 1. Figure 2 shows a global view of all MeHg-induced changes for chemically identified metabolites.

Table 1. Top 50 features identified by One-way ANOVA and post-hoc analysis

	Peaks(mz/rt)	p.value	-log10(p)	Post-hoc (Fisher's LSD)
1	2-aminoadipic acid	0e+00	28.45163	3 - 0; 3 - 1
2	308222	0e+00	23.15972	0 - 3; 1 - 3
3	2-ketoisocaproic acid	0e+00	22.04338	1 - 0; 3 - 0; 3 - 1
4	pyrazine 2,5-dihydroxy NIST	0e+00	21.91775	1 - 0; 3 - 0; 3 - 1
5	N-methylalanine	0e+00	17.69418	1 - 0; 3 - 0; 3 - 1
6	xanthine	0e+00	14.93298	1 - 0; 3 - 0; 3 - 1
7	citrulline	0e+00	14.63008	3 - 0; 3 - 1
8	benzylalcohol	0e+00	14.32162	0 - 1; 0 - 3; 1 - 3
9	glutamine	0e+00	12.31673	1 - 0; 3 - 0; 3 - 1
10	malic acid	0e+00	11.92368	1 - 0; 3 - 0; 3 - 1
11	267654	0e+00	11.39744	1 - 0; 3 - 0; 3 - 1
12	threonine	0e+00	11.16000	1 - 0; 3 - 0; 3 - 1
13	glycocyamine	0e+00	10.81091	3 - 0; 3 - 1
14	fumaric acid	0e+00	10.78489	1 - 0; 3 - 0; 3 - 1
15	proline	0e+00	10.69617	3 - 0; 3 - 1
16	4-hydroxymandelic acid	0e+00	9.87129	0 - 1; 0 - 3; 1 - 3
17	serine	0e+00	9.57658	3 - 0; 3 - 1
18	glycine	0e+00	9.34151	3 - 0; 3 - 1
19	inulobiose	0e+00	9.27370	0 - 3; 1 - 3
20	cysteine-glycine	0e+00	9.23519	1 - 0; 3 - 0; 3 - 1
21	glutaric acid	0e+00	9.15245	3 - 0; 3 - 1
22	histidine	0e+00	9.07598	3 - 0; 3 - 1
23	pantothenic acid	0e+00	8.65597	0 - 1; 0 - 3; 1 - 3
24	glycerol-3-galactoside	0e+00	8.36268	0 - 1; 0 - 3; 1 - 3
25	308189	0e+00	8.23416	0 - 1; 0 - 3; 1 - 3
26	2-hydroxyvaleric acid	0e+00	8.15908	1 - 0; 3 - 0
27	fructose	0e+00	8.09331	0 - 1; 0 - 3; 1 - 3
28	267805	0e+00	8.02260	1 - 0; 3 - 0; 3 - 1
29	nicotinamide	0e+00	7.98831	0 - 1; 0 - 3; 1 - 3
30	2,3-dihydroxybutanoic acid NIST	0e+00	7.56612	1 - 0; 3 - 0; 1 - 3
31	hippuric acid	0e+00	7.37565	0 - 1; 0 - 3; 1 - 3
32	alanine	0e+00	7.22462	3 - 0; 3 - 1
33	isoleucine	0e+00	6.97827	3 - 0; 3 - 1
34	216427	0e+00	6.91253	0 - 3; 1 - 3
35	maltotriose	0e+00	6.60142	0 - 3; 1 - 3
36	308203	0e+00	6.25035	0 - 3; 1 - 3
37	tyramine	0e+00	6.17016	3 - 0; 3 - 1
38	succinic acid	0e+00	5.83795	1 - 0; 3 - 0
39	2-deoxytetronic acid	0e+00	5.74507	1 - 0; 3 - 0; 3 - 1
40	elaidic acid	0e+00	5.62897	3 - 0; 3 - 1
41	232092	0e+00	5.51534	0 - 3; 1 - 3
42	308219	0e+00	5.48149	0 - 3; 1 - 3
43	tyrosine	0e+00	5.41677	3 - 0; 3 - 1
44	308113	0e+00	5.40190	1 - 0; 3 - 0
45	3-hydroxy-3-methylglutaric acid	0e+00	5.31799	3 - 0; 3 - 1
46	2-ketoadipic acid	1e-05	5.25669	0 - 3; 1 - 3
47	glucoheptulose	1e-05	5.13570	0 - 3; 1 - 3
48	indole-3-lactate	1e-05	5.10182	0 - 1; 0 - 3
49	homoserine	1e-05	5.02932	3 - 0; 3 - 1
50	2-hydroxy-2-methylbutanoic acid	1e-05	4.96289	0 - 3; 1 - 3

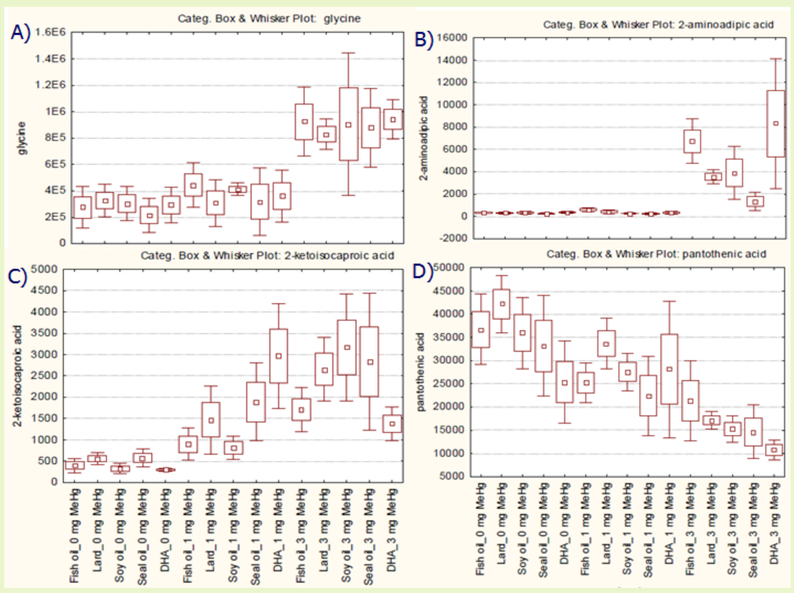

Figure 1. Mean intensities of peaks corresponding to glycine (A), 2-aminoadipic acid (B), 2-ketoisocaproic acid (C) and pantothenic acid (D) in rat urine samples.

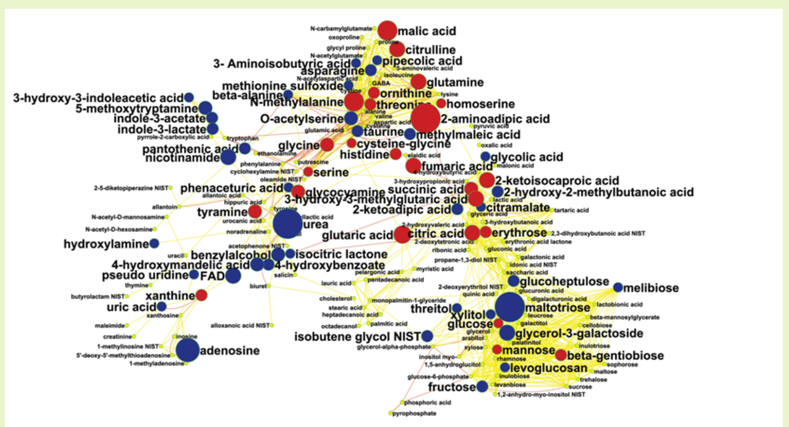

Figure 2. Metabolomic network diagram of 176 chemically identified metabolites. Metabolites were mapped by structural similarity (PubChem). Compounds enriched (●) or decreased (●) in urine samples of rats in the fish oil - 3 mg MeHg/Kg bw group vs fish oil controls. Node size is proportional to the relative change in intensities.

Application of the Random Forest method lead to the identification of 15 important features that best explain differences between MeHg dose groups (Figure 3).

DISCUSSION

This is the first study to our knowledge to adopt a metabolomic approach in order to identify biomarkers of MeHg exposure and effects.

Several potential urinary biomarkers were identified including metabolites related to amino acid degradation (2-ketoisocaproic acid, 2-aminoadipic acid, fumaric acid), urea cycle (citrulline, glutamine) and glutathione metabolism (cysteinyl-glycine).

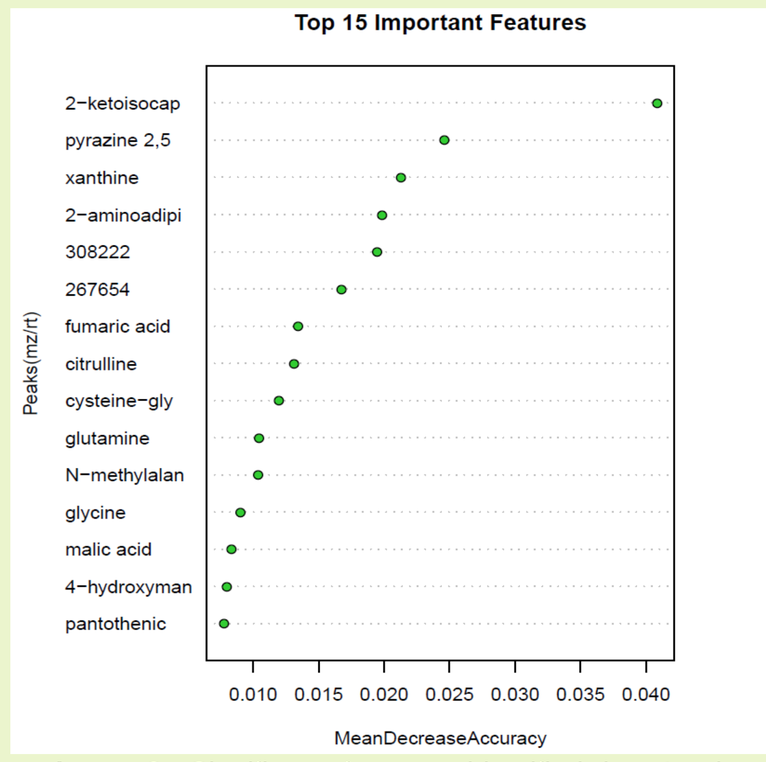

Figure 3. Significant features identified by Random Forest. The features are ranked by the mean decrease in classification accuracy when they are permuted.

CONCLUSION

A metabolomic approach was successfully applied for the identification of potential biomarkers of MeHg exposure. We are currently applying a similar analytical procedure to compare urinary metabolic profiles between Native Americans with low and high MeHg exposure.

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