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# Knockout of arsenic (+3 oxidation state) methyltransferase results in sex-dependent changes in phosphatidylcholine metabolism in mice

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## Abstract

Arsenic (+3 oxidation state) methyltransferase is the key enzyme in the methylation pathway for inorganic arsenic. We have recently shown that *As3mt* knockout (KO) has a profound effect on metabolomic profiles in mice. Phosphatidylcholine species (PCs) were the largest group of metabolites altered in both plasma and urine. The present study used targeted analysis to investigate the KO-associated changes in PC profiles in the liver, the site of PC synthesis. Results show that *As3mt* KO has a systemic effect on PC metabolism and that this effect is sex dependent.

### Keywords

Arsenic; *As3mt* knockout; Phophatidycholine; Mouse; Liver; Plasma

## Introduction

Inorganic arsenic (iAs) exposure through drinking water affects millions of people worldwide and is implicated in multiple cancers as well as non-cancer diseases. In humans, iAs is enzymatically methylated to mono- and dimethylated metabolites that are excreted mainly in urine (Thomas et al. 2007). Methylation of iAs is catalyzed by arsenic (+3 oxidation state) methyltransferase (AS3MT). Polymorphisms in AS3MT have been found to be associated with altered iAs metabolism and differing risks of cancer and metabolic disease (Antonelli et al. 2014). Thus, the *As3mt*-knockout (KO) mouse that cannot methylate iAs is a useful model for assessing the role of iAs metabolism in disease associated with iAs exposure. However, apart from the inability to methylate arsenic, the metabolism of the *As3mt*-KO mice has been poorly characterized.

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Recently, we published a study comparing the urinary and plasma metabolomes of C57BL/6 mice (WT) and *As3mt*-KO mice (Huang et al. 2016), with and without exposure to iAs. While we found few metabolites to be changed due to iAs treatment in either mouse strain, we found more significantly changed metabolites in comparisons of WT and KO animals, particularly among phosphatidylcholines (PCs) and other metabolites related to lipid metabolism.

PCs are primary components of cellular membranes, are precursors to signaling molecules, and make up 60–80 % of the phospholipid component of plasma lipoproteins (Cole et al. 2012). All nucleated cells synthesize PCs, but the liver is a major site for PC synthesis and plasma lipoprotein assembly (Cole et al. 2012). Notably, methylation of iAs occurs primarily in the liver (Watanabe and Hirano 2013), making it a target organ for iAs toxicity. The liver of *As3mt*-KO mice is particularly susceptible because it accumulates iAs (Currier et al. 2016; Hughes et al. 2010). To further probe the effects of *As3mt* KO on PC metabolism, we measured concentrations of molecular PC species in livers from mice in our recent metabolomic study and compared hepatic PCs profiles with those previously found in plasma.

## Materials and methods

### Animals and treatment

In the metabolomic study, adult male and female C57BL/6J WT and *As3mt*-KO mice were exposed to 0 and 1 ppm As as arsenite in drinking water for 4 weeks ($N = 15–21$/group) (Huang et al. 2016). Right medial lobes of the livers were collected at killing, snap-frozen in liquid nitrogen, and stored at −80 °C until analyzed.

### PC analysis

Liver tissue homogenate (150 μg protein) was extracted as previously described (Bligh and Dyer 1959) except substituting dichloromethane for chloroform. An internal standard 1,2-dimyristoyl-sn-glycero-3-phosphocholine (1.0 μg, Avanti Polar Lipids, Alabaster, AL) was added to the organic layer, and an aliquot (10 %) was injected into a tandem quadrupole mass spectrometer for continuous electrospray ionization (positive ions). Precursor ions of *m/z* 184 were measured for analysis of PC molecular species (Han et al. 2012) and quantitated using LipidView (Simons et al. 2012). Signals for molecular species of PC were ratioed to the signal from the internal standard.

### Data analysis

The relative concentrations of PC species were analyzed as described in our metabolomic study, using Partek for hierarchical clustering analysis and for ANCOVA analysis with sex, genotype, and iAs treatment as covariates (Huang et al. 2016). A $p$ value cutoff at $p < 0.05$ and a false-discovery rate $q$ value cutoff of $q < 0.1$ were used to determine PC species significantly changed due to genotype, sex or iAs treatment.

## Results and discussion

Twenty-nine PC species were identified in livers from all treatment groups. These species clustered primarily due to sex, followed by genotype, and not due to iAs treatment (Suppl. Figure 1). There were only 1–3 significantly changed hepatic PCs in comparisons of 0 ppm (untreated) and 1 ppm groups (Suppl. Table 1). These results are consistent with the major effect of sex and only minor effects of iAs exposure on plasma and urine metabolomes found in our metabolomic study (Huang et al. 2016).

*As3mt* KO changed greater numbers of hepatic PCs than did iAs exposure, but the extent of these changes differed between male and female mice. In untreated mice, more hepatic PCs were changed due to KO in male as compared to female mice (12 vs. 4), and this trend was also seen in iAs-treated mice (9 vs. 8) (Fig. 1a). Conversely, in the plasma, more PCs were changed due to *As3mt* KO in female as compared to male mice in both unexposed (48 vs. 14) and exposed groups (16 vs. 12) (Fig. 1b). In addition, *As3mt* KO affected different PCs in males and different PCs in females. Only one hepatic PC, PC (38:3), was increased due to KO in both male and female untreated mice; in iAs-treated mice, two PCs were changed—PC (38:3) increased and PC (32:0) decreased—in both males and females (Figs. 1a, 2). In plasma, only eight PCs were changed in both untreated males and females due to KO and only five PCs in iAs-treated mice (Fig. 1b). The fold change of PC concentrations in KO mice did not exceed 1.6; however, great numbers of PCs in both liver and plasma were affected (Fig. 1; Suppl. Table 2). Taken together, these results suggest that *As3mt* KO has a major effect on PC metabolism and that this effect is sex dependent.

The KO- or sex-associated changes in hepatic and plasma PC profiles may reflect alterations in hepatic PC synthesis (Vance 2008), export (Robins et al. 1991), or in PC remodeling (Hermansson et al. 2011), as well as in use of PCs as precursors to signaling molecules (Cole et al. 2012). Any combination of these processes could underlie the observed changes in PC profiles. Notably, in all of the KO vs WT comparisons, some PC species were significantly changed in both plasma and liver (Table 1). In particular, PC (38:3) was found changed (increased) due to KO in both plasma and liver of both untreated and iAs-treated male and female mice (Fig. 2). Future studies should examine the fatty acid tail composition and metabolism of PC (38:3) in iAs-exposed mice. With further characterization, this PC species may serve as a biomarker of iAs exposure or of iAs methylation capacity.

In conclusion, we found differences in hepatic PCs of WT and *As3mt-KO* mice that paralleled our previous findings in urinary and plasma metabolomes. While only minor differences in hepatic PCs were linked with iAs treatment, major changes in PCs were associated with *As3mt* KO, in a sex-specific manner. Notably, some *As3mt*-KO-associated PCs were found in both plasma and liver, suggesting that the effects of *As3mt* KO on PCs are systemic. These results are a further step in understanding the role iAs metabolism plays in adverse effects of iAs exposure.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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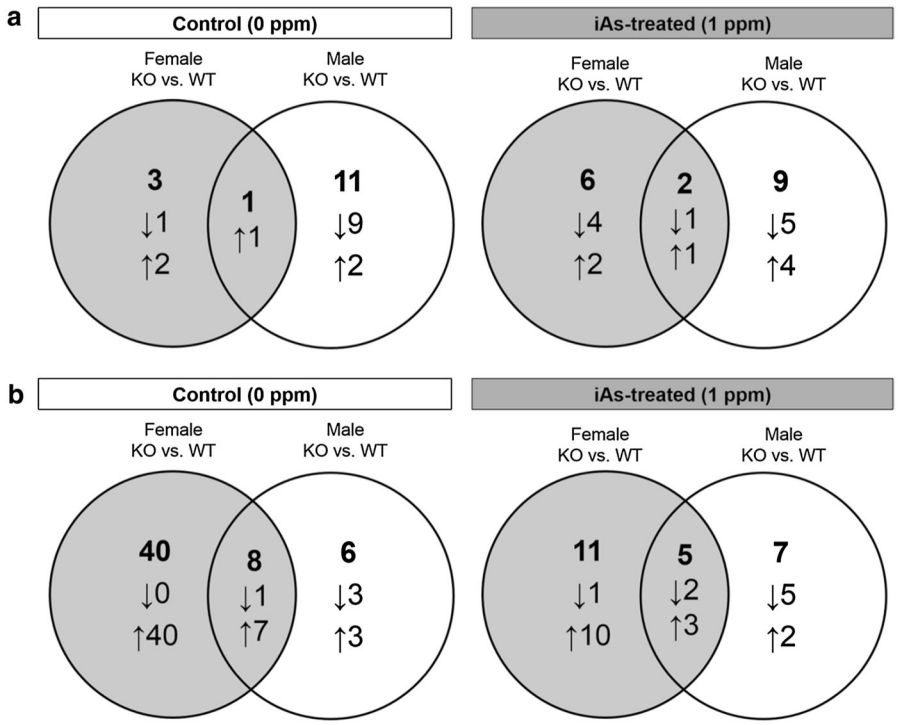


**Fig. 1.**

Number of hepatic (**a**) and plasma (**b**) PC species significantly changed ($p < 0.05$) in comparisons of *As3mt*-knockout (KO) and wild-type (WT) control and iAs-treated mice. *Bolded numbers* indicate total number of PCs changed in each comparison, for the compartment; *arrows* indicate increase or decrease of PCs in KO mice

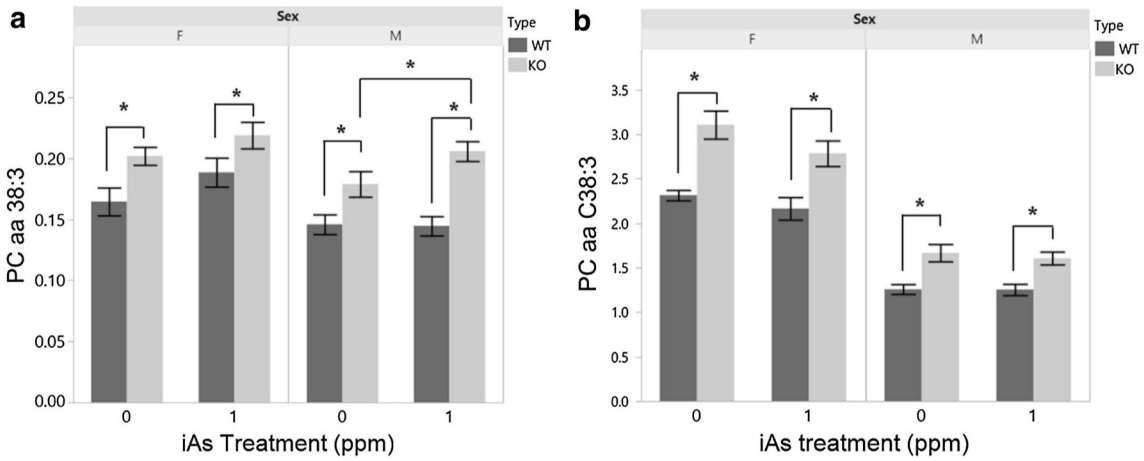


**Fig. 2.**

Relative abundance of the phosphatidylcholine (PC) species (C38:3 in liver (**a**) and plasma (**b**) of wild-type (WT) and *As3mt*-knockout (KO), untreated (0 ppm) and iAs-treated (1 ppm), male (M) and female (F) mice; *$p < 0.05$ and $q < 0.01$

## Table 1

Phosphatidylcholine (PC) species significantly changed in comparisons of *As3mt*-knockout (KO) and wild-type (WT) mice in both plasma and liver

| Comparison | PC species[a] | Fold change[b] in plasma | Fold change in liver |
|---|---|---|---|
| Untreated females KO versus WT | PC (C38:3) | 1.34 | 1.23 |
| | PC (C38:4) | 1.27 | 1.12 |
| | PC (C40:4) | 1.26 | 1.50 |
| Untreated males KO versus WT | PC (C32:1) | 1.47 | 1.21 |
| | PC (C38:3) | 1.33 | 1.22 |
| | PC (O-C40:6) | −1.25 | −1.30 |
| iAs-treated females KO versus WT | PC (C38:3) | 1.29 | 1.16 |
| | PC (C36:2) | 1.17 | 1.21 |
| iAs-treated males KO versus WT | PC (C32:1) | 1.51 | 1.35 |
| | PC (C36:5) | 1.16 | 1.19 |
| | PC (C38:3) | 1.29 | 1.42 |
| | PC (C40:4) | 1.23 | 1.26 |
| | PC (O-C36:2) | −1.18 | −1.36 |
| | PC (O-C40:6) | −1.49 | −1.36 |

[a]Phosphatidylcholine notation: PC(X:Y) indicates a phosphatidylcholine X total carbons and Y double bonds. PC(O-X:Y) indicates a phosphatidylcholine with one acyl and one ester linkage to the fatty acid tails with X total carbons and Y double bonds

[b]Fold change indicates an increase or decrease in KO animals