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Serum homocysteine, arsenic methylation, and arsenic-induced skin lesion incidence in Bangladesh: A one-carbon metabolism candidate gene study

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ABSTRACT

Background: Inorganic arsenic (As) is methylated via one carbon metabolism (OCM) to mono- and dimethylated arsenicals (MMA and DMA), facilitating urinary excretion. Hyperhomocysteinemia (HHcys), a marker of impaired OCM, is a risk factor for As-induced skin lesions, but the influences of single nucleotide polymorphisms (SNPs) in OCM genes on Hcys, As metabolism and skin lesion risk is unclear.

Objectives: To (i) explore genetic sources of Hcys and the causal role of HHcys in As-induced skin lesion development using OCM genetic proxies for HHcys and (ii) identify OCM SNPs associated with urinary As metabolite proportions and/or skin lesion incidence.

Methods: We conducted a case-control study nested in the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh which 876 incident skin lesion cases were matched to controls on sex, age, and follow-up time. We measured serum Hcys, urinary As metabolites, and 26 SNPs in 13 OCM genes.

Results: Serum Hcys and urinary %DMA were independently associated with increased and decreased odds of skin lesions, respectively. The T allele of *MTHFR* 677 C➔T (rs1801133) was associated with HHcys, higher %MMA, and lower %DMA, but not with skin lesions. Interactions between SNPs and water As on skin lesion risk were suggestive for three variants: the G allele of *MTRR* rs1801394 and T allele of *FOLR1* rs1540087 were associated with lower odds of skin lesions with lower As (≤50 μg/L), and the T allele of *TYMS* rs1001761 was associated with higher odds of skin lesions with higher As.

Conclusions: While HHcys and decreased %DMA were associated with increased risk for skin lesions, and *MTHFR* 677 C➔T was a strong predictor of HHcys, *MTHFR* 677 C➔T was not associated with skin lesion risk. Future studies should explore (i) non-OCM and non-genetic determinants of Hcys and (ii) if genetic findings are replicated in other As-exposed populations, mechanisms by which OCM SNPs may influence the dose-dependent effects of As on skin lesion risk.

1. Introduction

Elevated homocysteine (Hcys) in serum and/or plasma, a condition known as hyperhomocystenemia (HHcys), is a well-established risk factor for numerous health conditions, including cardiovascular disease (CVD) (Ganguly and Alam, 2015), neurologic conditions (Ansari et al.,

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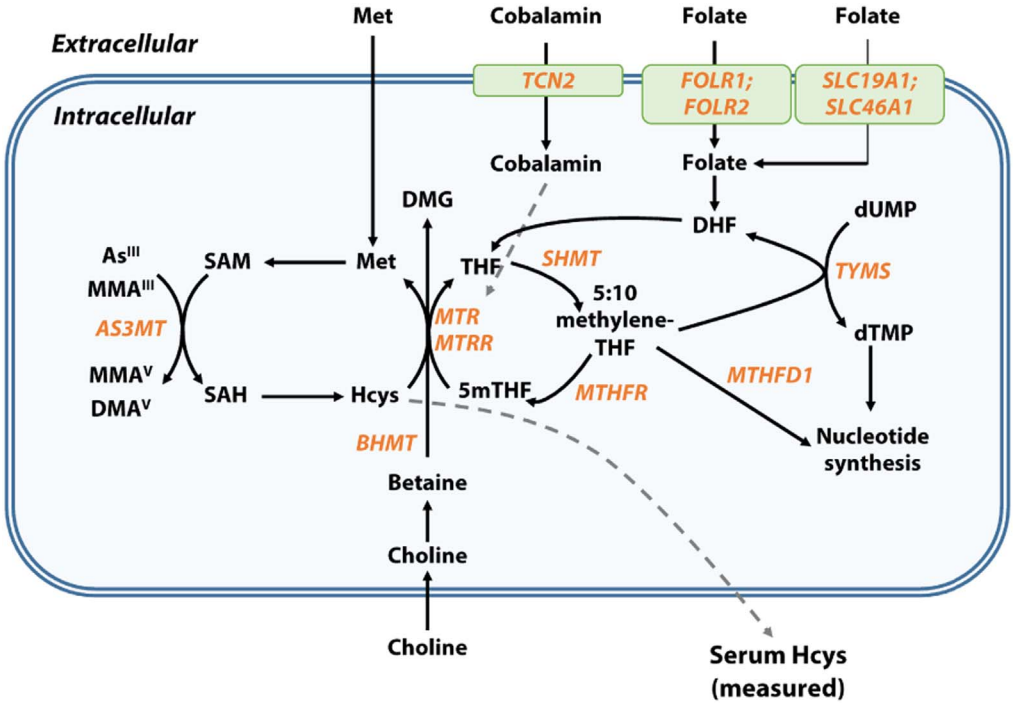

Fig. 1. One-carbon metabolism, arsenic methylation, and homocysteine. Folate enters the cell through receptor-mediated (folate receptor alpha [FRα], gene *FOLR1*; beta [FRβ], gene *FOLR2*; or gamma [FRγ]) or carrier-mediated (solute carrier family 19, member 1 [SLC19A1]) transport mechanisms. Folic acid is reduced to dihydrofolate (DHF) and tetrahydrofolate (THF). Serine hydroxymethyltransferase (SHMT) catalyzes the conversion of serine and THF to 5:10 methylene-THF, which can be used for nucleotide synthesis via methylenetetrahydrofolate dehydrogenase 1 (MTHFD) or thymidylate synthetase (TYMS). Alternatively, 5:10 methylene-THF can be converted to 5-methyl THF (5mTHF) by methylene tetrahydrofolate reductase (MTHFR). The methyl group from 5mTHF is transferred to Hcys via methionine synthetase (MTR), a cobalamin-dependent enzyme, which generates methionine (Met) and THF. Betaine can also serve as the methyl donor for the remethylation of Hcys to Met in a reaction catalyzed by betaine-homocysteine *S*-methyltransferase (BHMT). Met is activated to form S-adenosylmethionine (SAM), the methyl donor for the methylation of InAs and MMA, yielding MMA and DMA, respectively, and S-adenosylhomocysteine (SAH). SAH is hydrolyzed to regenerate Hcys, which can be remethylated to Met or directed toward the transsulfuration pathway by cystathionine-β-synthase (CBS). Excess intracellular Hcys can be exported extracellularly. Genes with SNPs examined in the current study are displayed in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2014), and cancer (Wu and Wu, 2002). Homocysteine is endogenously synthesized via B-vitamin-dependent one-carbon metabolism (OCM). As shown in Fig. 1, the donation of a methyl group from *S*-adenosylmethionine (SAM) to various substrates yields the methylated product and *S*-adenosylhomocysteine, which is hydrolyzed to form Hcys (Scott and Weir, 1998). HHcys is a sensitive indicator of B-vitamin deficiencies, especially for folate and cobalamin (Savage et al., 1994). Although Hcys induces oxidative stress and inflammation in experimental models (Dayal et al., 2004; Eberhardt et al., 2000), whether HHcys is a causative factor in human disease, or merely a biomarker of disease risk, remains under debate (Brattstrom and Wilcken, 2000).

Chronic exposure to arsenic (As) is associated with increased all-cause mortality (Argos et al., 2010) and elevated risk for a variety of conditions, such as CVD (Moon et al., 2012), neurological deficits (Tyler and Allan, 2014), and cancers of the skin, lung, bladder, liver, and kidney (Navarro Silvera and Rohan, 2007). Inorganic As is metabolized through a series of methylation reactions to monomethyl (MMA) and dimethyl (DMA) species (Fig. 1) by arsenite 3-methyltransferase (*AS3MT*), with methyl groups donated by SAM via OCM (Lin et al., 2002). An increased capacity to methylate As—as indicated by decreased proportions of inorganic As (InAs) and MMA and an increased proportion of DMA in urine—has been associated with reduced risk for several As-associated conditions (Steinmaus et al., 2007). Importantly, nutritional manipulation of OCM in randomized controlled trials (e.g., folic acid supplementation) has been shown to increase As methylation capacity, reduce total blood As concentrations, and reduce Hcys levels in As-exposed populations (Gamble et al., 2006; Gamble et al., 2007; Peters et al., 2015), suggesting the utility of public health interventions targeting OCM factors to reduce As toxicity.

A hallmark of chronic As exposure is the appearance of arsenical skin lesions, which are a sensitive indicator of increased risk for various As-related diseases (Karagas et al., 2015). As such, identifying risk factors for skin lesion development may provide insight into mechanisms of As toxicity and potential targets for intervention. Previously, our group found that HHcys and other factors associated with OCM status were associated with increased risk for As-induced skin lesions in Bangladesh (Pilsner et al., 2009), suggesting that compromised OCM might contribute to increased susceptibility to As toxicity, possibly by impacting As methylation capacity. Alternatively, HHcys may act as a biomarker reflecting dysregulation of other components of the OCM network that contribute to increased susceptibility to As, e.g., through nucleotide biosynthesis and its impact on DNA repair (Locasale, 2013).

While Hcys levels are known to be influenced by single nucleotide polymorphisms (SNPs) in several genes involved in OCM, the most widely-studied variant is the nonsynonymous 677 C➔T polymorphism (rs1801133) in methylenetetrahydrofolate reductase (*MTHFR*). *MTHFR* encodes for an enzyme that synthesizes 5-methyltetrahydrofolate (5mTHF), a substrate required for the remethylation of Hcys to methionine. *MTHFR* 677 C➔T is a nonsynonymous SNP associated with reduced enzyme activity, thereby resulting in elevated Hcys, particularly in populations with low folate intakes (Jacques et al., 1996). Since the 677 C➔T variant is causally associated with HHcys, it is commonly used as a genetic proxy for HHcys to examine the causal nature of HHcys-disease associations (Clarke et al., 2012; Wald et al., 2002).

The prevalence of HHcys is particularly high among individuals of South Asian descent, which has been hypothesized to reflect folate and cobalamin deficiencies and genetic factors (Chandalia et al., 2003; Senaratne et al., 2001). A previous cross-sectional survey from our group observed a high prevalence of HHcys in Bangladesh, and plasma folate and cobalamin was found to explain 15% and 5% of the variance in plasma Hcys, respectively (Gamble et al., 2005). It is unknown whether *MTHFR* 677 C➔T and other one-carbon metabolism gene variants are associated with Hcys concentrations in Bangladesh: these SNPs have variable effects on Hcys concentrations across populations (Wald et al., 2012), and the associations of these genetic variants with Hcys have not been examined previously in this population.

Several studies have investigated the modulating effects of OCM gene variants on As-related health outcomes, including skin lesions (Ahsan et al., 2007; Seow et al., 2015), bladder cancer (Beebe-Dimmer et al., 2012; Chung et al., 2010; Karagas et al., 2005), breast cancer (Gamboa-Loira et al., 2017), elevated blood pressure (Farzan et al., 2015), and myelomeningocele (Mazumdar et al., 2015), though findings have been mixed. OCM gene variants have also been linked to altered uAs metabolite patterns in Argentina (Engstrom et al., 2011;