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Supplemental Material

Arsenic Metabolism in Mice Carrying a *BORCS7/AS3MT* Locus Humanized by Syntenic Replacement

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Figure S2. Expression of *BORCS7-AS3MT* read-through transcripts in tissues of the humanized (Hs/Hs) and wild type (WT/WT) mice and the WT/Hs heterozygotes. **A.** Read-through transcript expression in testes, adrenal glands, and liver of Hs/Hs mice (N=1/tissue). **B.** Separation of cDNA from testes of WT/WT, WT/Hs and Hs/Hs mice on a 1.6% agarose gel (N=1/genotype): (a) 100 bp fragment, consistent with the previously described read-through transcript; (b) 220 bp fragment of a read-through transcript that has not been previously reported. **C.** Schematic structures of the unspliced *BORCS7* isoforms (lavender), *AS3MT* (blue) and the previously reported (orange) (Lu X et al. 2015) and novel (red) *BORCS7-AS3MT* read-through transcripts. Introns are indicated by thin lines, untranslated exonic sequence by medium lines, and exonic coding sequence by thick lines. The position of the stop codon is indicated for the top two *BORCS7* transcripts. The PCR used for quantification and sequencing of the *BORCS7-AS3MT* junction of the read-through transcripts is indicated at the bottom of the figure (green).

Figure S3. Concentrations of inorganic arsenic (iAs), methyl-arsenic (MAs) and dimethyl-arsenic (DMAs) (µg As/L) in urine of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs (20 µg As/ kg body weight). Mean (x), median (—), 25th and 75th percentiles (box), maximum and minimum (whiskers), and individual values including outliers are shown (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). MAs concentration was below limit od detection in 52 out of 54 urine samples collected from WT/WT mice during the 3 collection intervals; a value of 0 µg As/L was imputed for MAs concentrations in these samples. ^{a,b,c} Within each panel, statistically significant differences between strains and sexes are marked with different letters. (ANOVA with Student-Newman-Keuls post-test.).

Figure S4. Proportions of total arsenic (%tAs) represented by inorganic arsenic (%iAs), methyl-arsenic (%MAs) and dimethyl-arsenic (%DMAs) in urine of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs (20 µg As/kg body weight). Mean +SD (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). MAs concentration was below limit od detection in 52 out of 54 urine samples collected from WT/WT mice during the 3 collection intervals; a value of 0 µg As/L was imputed for MAs Within each panel, statistically significant differences between strains and sexes are marked with different letters: ^{a,b,c} for differences in %iAs, ^{d,e,f} for differences in %MAs, g,hi for differences in %DMAs. (ANOVA with Student-Newman-Keuls post-test.).

Figure S5. Concentrations of inorganic arsenic (iAs), methyl-arsenic (MAs) and dimethyl-arsenic (DMAs) (µg As/L) in feces of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs (20 µg As/ kg body weight). Mean (x), median (—), 25th and 75th percentiles (box), maximum and minimum (whiskers), and individual values including outliers are shown (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). DMAs concentration was below limit of detection in 48 out of 54 fecal samples collected during the 3 collection intervals from male and female Hs/Hs mice; a value of 0 µg As/kg was imputed for DMAs concentrations in these samples. ^{a,b} Within each panel, statistically significant differences between strains and sexes are marked with different letters. (ANOVA with Student-Newman-Keuls post-test.).

Figure S6. Proportions of total arsenic (%tAs) represented by inorganic arsenic (%iAs), methyl-arsenic (%MAs) and dimethyl-arsenic (%DMAs) in feces of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 24-h intervals after oral administration of a single dose of iAs (20 μg As/kg body weight). Mean +SD (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). DMAs concentration was below limit of detection in 48 out of 54 fecal samples collected during the 3 collection intervals from male and female Hs/Hs mice; a value of 0 μg As/kg was imputed for DMAs concentrations in these samples. Within each panel, statistically significant differences between strains and sexes are marked with different letters: ^{a,b,c} for differences in %iAs, ^{d,e,f,g} for differences in %MAs, ^{h,i,j} for differences in %DMAs. (ANOVA with Student-Newman-Keuls post-test.).

Figure S7. Proportions of total arsenic (%tAs) represented by inorganic arsenic (%iAs), methyl-arsenic (%MAs) and dimethyl-arsenic (%DMAs) in urine of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice collected during 4-week exposure to iAs in drinking water (400 μg As/L). Mean +SD (N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). MAs concentration was below LOD in 70 out of 72 urine samples collected from WT/WT mice during the 4 collection intervals; a value of 0 μg As/L was imputed for MAs concentrations in these samples. Within each panel, statistically significant differences between strains and sexes are marked with different letters: ^{a,b,c} %iAs, ^{d,e,f} %MAs, ^{g,h} %DMAs. (ANOVA with Student-Newman-Keuls post-test.).

Figure S8. Concentrations of total arsenic (μg As/kg) in livers and kidneys of humanized (Hs/Hs) and wild type (WT/WT) male (M) and female (F) mice after 4-week exposure to iAs in drinking water (400 μg As/L). Total arsenic was calculated as sum of inorganic arsenic, methyl-arsenic and dimethyl-arsenic. Mean (x), median (—), 25th and 75th percentiles (box), maximum and minimum (whiskers), and individual values including outliers are shown (Mean+SD; N=8 for Hs/Hs males, N=10 for Hs/Hs females, N=7 for WT/WT males, and N=11 for WT/WT females). DMAs was below LOD in 16 out of 18 liver samples collected from Hs/Hs mice; a value of 0 μg As/kg was imputed for DMAs concentrations in these samples. ^{a,b,c} Within each panel, statistically significant differences between strains and sexes are marked with different letters. (ANOVA with Student-Newman-Keuls post-test.).

References