Supporting Information

Gender-specific Effects of Arsenic Exposure on the Trajectory and Function of the Gut Microbiome

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Supplemental Materials and Methods

Animals, Arsenic Exposure and samples collection. Sodium arsenite were obtained from Fisher Scientific (Pittsburgh, PA). 40 SPF grade C57BL/6 female and male mice mice (~8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME). These mice are divided to four groups: group A: 10 female mice as control group; group B: 10 female mice which are treated with 10 ppm arsenic through drinking water; group C: 10 male mice as control group; group D: 10 male mice which are treated with 10 ppm arsenic through drinking water. We reloaded the drinking water once a week to avoid oxidation of sodium arsenite. Mice were provided standard pelleted rodent diet (PicoLab Rodent Diet 20). Arsenic exposure time is 4 weeks and we collected fecal pellets before exposure and after 4-week exposure. Mice are maintained in University of Georgia animal facility throughout the duration of the experiment in static microisolator cages with Bed-O-Cob combination bedding under environmental conditions of 22°C, 40–70% humidity, and a 12:12 hr light:dark cycle. All experiments were approved by the UGA Committee on Animal Care. All mice are treated humanely as approved in the animal protocol.

16S rRNA gene sequencing. We isolated DNA from fecal pellets by using a PowerSoil® DNA Isolation Kit as instructed by the manufacturer (MO BIO Laboratories, CA). DNA was amplified using universal primers iTRU-A 515 F and iTRU-1 806 R to target the V4 regions of 16S rRNA of bacteria. Individual samples were barcoded, pooled to construct the sequencing library, and sequenced (Illumina Miseq) to generate pair-ended 300 x 300 reads.

Analysis of 16S rRNA sequencing data. All of sequences files were preliminarily treated with Geneious R8. Two sequencing files of each samples were set paired, and the primers regions were annotated and trimmed (the error probability limit were set to 0.01). Paired reads then were merged together. The output fasta files were further analyzed by Quantitative Insights into Microbial Ecology (QIIME).[1] Operational Taxonomic Units (OTUs) were annotated and classed. UCLUST was used to choose the Operational Taxonomic Units (OTUs) with a threshold of 97% sequence similarity. A representative set of sequences from each OTU was selected for taxonomic identification of each OTU using the Ribosomal Database Project (RDP) classifier. The latest Greengenes OTUs reference sequences (97% sequence similarity) were used as the training sequences for RDP.[2] Our analyses were typically conducted at the genus level due to higher confidence in the assignment of taxa based on the sequencing reads.

Analysis of metagenomics data. Metagenomics sequencing data (pair-ended 150 x 150 reads) were analyzed with the MG-RAST pipeline for further annotation and functional analysis.[3]

Statistical analysis. Multivariate statistical methods were used to compare gut microbiome communities between groups. Principal coordinate analysis (PCoA) was performed to examine intrinsic clusters within the observations with the β-diversity as the metrics. And, Jackknifed β-diversity and hierarchical clustering analysis via Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to differentiate the gut microbiome profiles of the controls and arsenic-treated mice. The difference in the individual gut microbiome composition between control and arsenic-treated groups was assessed using a non-parametric test with Metastats as described previously.[4] Student t-test was used to compare the functional pathways between control group and arsenic-treated group in both gender mice.

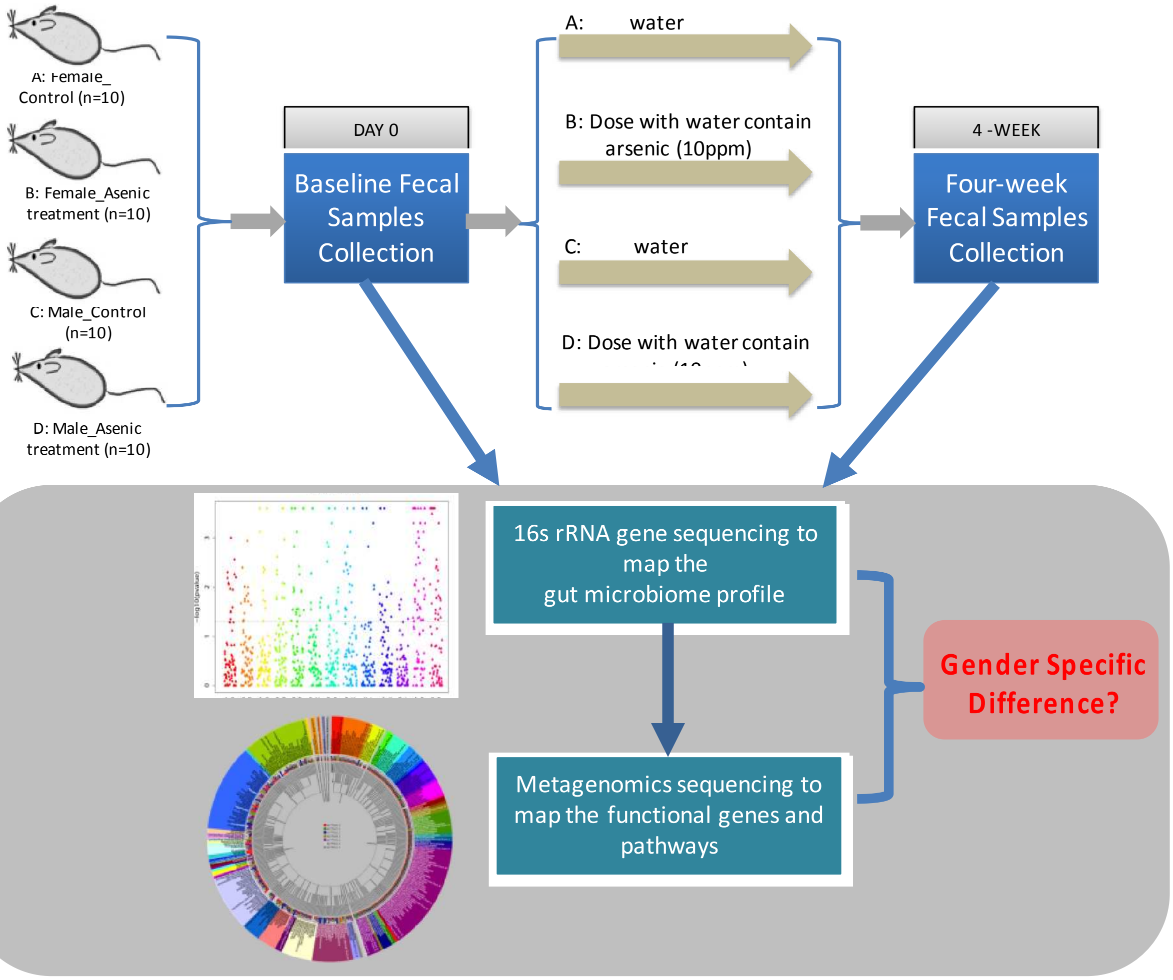

Figure S1. Experiment workflow. We combined 16S rRNA gene sequencing and metagenomics sequencing to explore the gender-specific effects of arsenic exposure on the trajectory and function of gut microbiome.

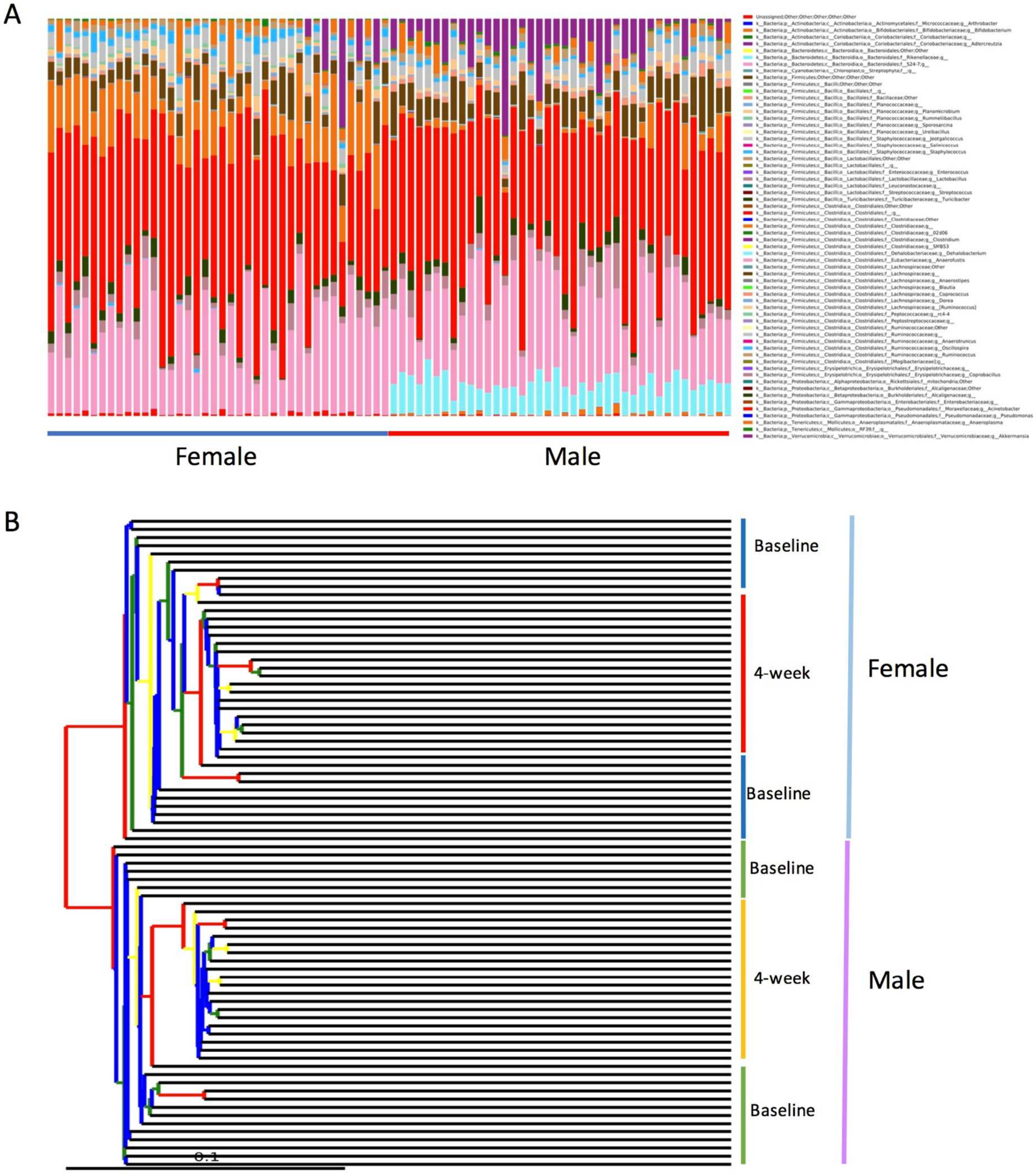

Figure S2. A. Gut microbiome composition profiles in male and female mice (k, bacteria; p, phylum; c, class; o, order; f, family; g, genus) as revealed by 16S rRNA sequencing. B. Hierarchical clustering analysis by UPGMA. Gut microbiome in female and male mice are clearly separated. Time-dependent clustering is also evident across all the samples analyzed.

Table S1. Significantly different gut bacteria genera between the baseline and 4-week samples in female mice (controls and arsenic treated animals)

Taxa	Control Group				Arsenic Group			
	Mean (Baseline)	Mean (4 Weeks)	Fold change	P- value	Mean (Baseline)	Mean (4 Weeks)	Fold change	P- value
F1	0.25255	0.216032	-1.17	0.380619	0.292919	0.180785	-1.62	0.004995
F2	0.00002	0	—	0.009136	0	0	—	1
F3	0.000015	0	—	0.009136	0	0	—	1
F4	0.000407	0	—	0.000999	0	0	—	1
F5	0.000016	0	—	0.019981	0	0	—	1
F6	0.004386	0	—	0.000999	0.000027	0.000003	-9	0.015654
F7	0.000095	0	—	0.037962	0	0	—	1
F8	0.01685	0.035109	2.08	0.002997	0.014297	0.024568	1.72	0.035964
F9	0.001134	0.001874	1.65	0.025974	0.001595	0.000775	-2.06	0.017982
F10	0.000355	0.001155	3.25	0.005994	0.000543	0.000917	1.69	0.134865
F11	0.000208	0.000596	2.87	0.015984	0.000098	0.000577	5.89	0.000999
F12	0.005673	0.006459	1.14	0.375624	0.002781	0.005731	2.06	0.013986
F13	0.007925	0.00376	-2.11	0.00999	0.008475	0.003941	-2.15	0.006993
F14	0.018699	0.014633	-1.28	0.252747	0.008613	0.017585	2.04	0.01998
F15	0.004203	0.008448	2.01	0.006993	0.005247	0.006661	1.27	0.235764
F16	0.027287	0.042622	1.56	0.008991	0.028024	0.051027	1.82	0.00999
F17	0.014615	0.010669	-1.37	0.21978	0.007857	0.013081	1.67	0.034965
F18	0.00007	0.000327	4.67	0.008991	0.00008	0.000405	5.06	0.00999
F19	0.000159	0.000006	-26.5	0.003996	0.00022	0.000024	-9.17	0.000999
F20	0.000034	0	—	0.040959	0	0	—	1
F21	0.00003	0	—	0.013986	0	0	—	1
F22	0.000018	0.00002	1.11	1	0.000009	0.066169	7352	0.001998

*The abbreviations of bacteria genus are listed as below (k, bacteria; p, phylum; c, class; o, order; f, family; g, genus):

F1: *k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;g__;*
F2: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other;Other;*
F3: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Rummeliibacillus;*
F4: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Jeotgalicoccus;*
F5: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__;*
F6: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus;*
F7: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina;*
F8: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter;*
F9: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;Other;*
F10: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;*
F11: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes;*
F12: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus;*
F13: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea;*
F14: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Ruminococcus;*
F15: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__rc4-4;*
F16: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__;*
F17: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;*
F18: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Mogibacteriaceae;g__;*
F19: *k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;Other;*
F20: *k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__;*
F21: *k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;*
F22: *k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia;*

Table S2. Significantly different gut bacteria genera between the baseline and 4-week samples in male mice (controls and arsenic treated animals)

Taxa	**Control Group**				**Arsenic Group**			
	Mean (Baseline)	**Mean(4 Weeks)**	**Fold change**	**P- value**	**Mean (Baseline)**	**Mean(4 Weeks)**	**Fold change**	**P- value**
M1	0.001231	0.005125	4.16	0.004995	0.000673	0.010491	15.59	0.000999
M2	0	0.000013	—	0.037087	0	0.000011	—	0.135126
M3	0.000566	0.000694	1.23	0.503497	0.000272	0.000829	3.05	0.000999
M4	0.000004	0.000018	4.5	0.049589	0	0.000017	—	0.037649
M5	0	0.000776	—	0.001998	0	0.000031	—	0.003996
M6	0.025357	0.037924	1.50	0.101898	0.019862	0.054252	2.73	0.012987
M7	0.000115	0.000165	1.43	0.186813	0.000078	0.000177	2.27	0.033966
M8	0.00679	0.039658	5.84	0.000999	0.010739	0.032304	3.01	0.013986
M9	0.000003	0	—	0.444542	0.000016	0	—	0.003294
M10	0.00089	0.00162	1.82	0.004995	0.001047	0.001322	1.26	0.21978
M11	0.002452	0.003219	1.31	0.286713	0.001557	0.004104	2.64	0.000999
M12	0.000017	0	—	0.007717	0.000036	0.000021	-1.71	0.456543
M13	0.014339	0.012032	-1.19	0.292707	0.014443	0.022266	1.54	0.041958
M14	0.000175	0.000416	2.38	0.018981	0.000212	0.000539	2.54	0.003996
M15	0.035263	0.007172	-4.92	0.011988	0.025311	0.00433	-5.85	0.000999
M16	0.003216	0.007536	2.34	0.002997	0.003129	0.004885	1.56	0.103896

*The abbreviations of bacteria genus are listed as below (k, bacteria; p, phylum; c, class; o, order; f, family; g, genus):

M1: *k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales; f__Bifidobacteriaceae;g__Bifidobacterium*
M2: *k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae; g__*
M3: *k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae; g__Adlercreutzia*
M4: *k__Bacteria;p__Firmicutes;Other;Other;Other;Other*
M5: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus*
M6: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae; g__Lactobacillus*
M7: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;*

g__Streptococcus

M8: *k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae; g__Turicibacter*

M9: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__SMB53*

M10: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae; g__Dehalobacterium*

M11: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea*

M12: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__*

M13: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae; g__Ruminococcus*

M14: *k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Mogibacteriaceae;g__*

M15: *k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae; g__Anaeroplasma*

M16: *k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__;g__*

Reference

(1) Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., and Gordon, J. I. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods 7*, 335-336.

(2) Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol. 73*, 5261-5267.

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(4) White, J. R., Nagarajan, N., and Pop, M. (2009) Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Comput. Biol. 5*, e1000352.