Supplemental Material

Genome-Wide Association Study to Identify Genes Related to Renal Mercury Concentrations in Mice

Hammoudi Alkaissi, Jimmy Ekstrand, Aksa Jawad, Jesper Bo Nielsen, Said Havarinasab, Peter Soderkvist, and Per Hultman

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</table>
Microsatellite markers used in the Genome Wide-Scan study with an average spacing of 20 cM. Amplicon size for each microsatellite are presented for A/J and C57BL/6 strains. Forward and reverse primer sequences are presented for each microsatellite.

**Table S2.** Designed primers used for fine mapping

<table>
<thead>
<tr>
<th>Gene, Protein name</th>
<th>Symbols</th>
<th>Direction Primer Pairs</th>
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<tbody>
<tr>
<td>Peroxisome proliferative activated receptor, gamma, coactivator-related 1</td>
<td>Pprc1</td>
<td>F AACCTGACTAGCCCAGTCCT AGGACATCAGCTGGAGACAAG</td>
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<tr>
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<td></td>
<td>R AGGACATCAGCTGGAGACAAG</td>
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<tr>
<td>Beta-transducin repeat containing protein</td>
<td>Btrc</td>
<td>F GAAGGAAGAGGAGGCGGGAT CTCTGCCGGGTCCATAATCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R AAACCTGGACATTTCTCTTACTGGA CTCTGCCGGGTCCATAATCG</td>
</tr>
<tr>
<td>Nuclear factor of kappa light polypeptide gene enhancer in B cells 2, p49/p100</td>
<td>Nfkb2</td>
<td>F GAGAGAAGCTGTCTGGGTACG GAGAGAAGCTGTCTGGGTACG</td>
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<tr>
<td></td>
<td></td>
<td>R GATGCTTAGGTTGAGTGGGTT GAGGAAGAGGAGGCGGGAT</td>
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<td>Sec31 homolog B (S. cerevisiae)</td>
<td>Sec31b</td>
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<tr>
<td></td>
<td></td>
<td>R TGCGTCAGTGTCACATACC TCCAAGGGATCATTCCCTCCC</td>
</tr>
<tr>
<td>Progressive external ophthalmoplegia 1</td>
<td>Peo1</td>
<td>F GAGGAGCCTGCCACTTCTGCTGGTCTG</td>
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<td></td>
<td>R CATGGAAGAAAGATGCTCTTGTGGTCTG</td>
</tr>
<tr>
<td>Leucine zipper, putative tumor suppressor 2</td>
<td>Lzts2</td>
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<td>R CTTTCTTCTGCTGGTCTG</td>
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<td>PDZ domain containing 7</td>
<td>Pdzd7</td>
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<td></td>
<td>R CCTAGAGCGAAGATGGGGTCAGGT</td>
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<tr>
<td>T cell leukemia, homeobox 1</td>
<td>Tlx1</td>
<td>F AGCCCATACGCTTGCCATT</td>
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<tr>
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<td>R AGCCCATACGCTTGCCATT</td>
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Designed primers covering SNPs on genes selected for fine mapping study, since they are polymorph between background strain A/J and C57BL/6J. Forward and reverse primer sequences are presented for each marker.

**Table S3.** Microsatellite markers used for haplotyping

<table>
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<tr>
<th>Markers</th>
<th>Chr</th>
<th>cM</th>
<th>Direction</th>
<th>Primer Pairs</th>
<th>A</th>
<th>C57BL6</th>
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</table>
Microsatellite markers that differ between background strains A/J and C57BL/6, used in the haplotype study in order to narrow down the QTL region. Primer sequences and amplicon sizes of A/J and C57BL/6 are also presented.

**Table S4.** PCR primers amplified for Sanger Sequencing

<table>
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<tr>
<th>Gene</th>
<th>Direction</th>
<th>Primer Sequence</th>
<th>Amplicon Size</th>
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<td>Tlx1</td>
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<td>TGGGCAAGTCAAGTCTCCCA</td>
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<td>R</td>
<td>CTAACAGGCAAACGCTGCC</td>
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<td>Tlx1</td>
<td>F</td>
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<td>Tlx1</td>
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<td>R</td>
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</table>

Designed primer sequences for *Lbx1* and *Tlx1* covering exons for PCR amplification needed for Sanger sequencing. Primers cover SNPs that are predicted to be polymorph between A/J and C57BL/6 according to Ensembl database.

**Table S5.** Gene targets for Real-Time PCR

<table>
<thead>
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<th>Gene/Protein name</th>
<th>Gene Symbols</th>
<th>Species</th>
<th>Dye Label</th>
<th>Assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxisome proliferative activated receptor, gamma, coactivator-related 1</td>
<td><em>Pprc1</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm00521078_m1</td>
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<tr>
<td>Nuclear respiratory factor 1</td>
<td><em>Nrf1</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm01135606_m1</td>
</tr>
<tr>
<td>Nuclear factor, erythroid derived 2, like 2</td>
<td><em>Nfe2l2, Nrf2</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm00477784_m1</td>
</tr>
<tr>
<td>Beta-transducin repeat containing protein</td>
<td><em>Btrc</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm00477667_m1</td>
</tr>
<tr>
<td>Nuclear factor of kappa light polypeptide gene enhancer in B cells 2, p49/p100</td>
<td><em>Nfkb2</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm00479810_g1</td>
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<tr>
<td>Peptidylprolyl isomerase A (Cyclophilin A)</td>
<td><em>Ppia</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm02342430_g1</td>
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<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td><em>Gapdh</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm99999915_g1</td>
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</table>

Gene targets used in the gene expression analysis, measured with FAM labeled probes. Gene/protein names followed by gene symbols are presented.

**Table S6.** Potential housekeeping genes tested for Hg exposure

<table>
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<th>Gene/Protein name</th>
<th>Symbols</th>
<th>Species</th>
<th>Dye Label</th>
<th>Assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin beta</td>
<td><em>Actb</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm02619580_g1</td>
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<tr>
<td>Peptidylprolyl isomerase A (Cyclophilin A)</td>
<td><em>Ppia</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm02342430_g1</td>
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<tr>
<td>Phosphoglycerate kinase</td>
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<td>Mm00435617_m1</td>
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<td>Eukaryotic 18S ribosomal RNA</td>
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<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td><em>Gapdh</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm99999915_g1</td>
</tr>
<tr>
<td>Beta-2-microglobulin</td>
<td><em>B2m</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm00437762_m1</td>
</tr>
<tr>
<td>Transferrin receptor (P90, CD71)</td>
<td><em>Tfrc</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm00441941_m1</td>
</tr>
<tr>
<td>TATA box–binding protein</td>
<td><em>Tbp</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm01277042_m1</td>
</tr>
<tr>
<td>Hypoxanthine Phosphoribosyltransferase</td>
<td><em>Hprt</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm03024075_m1</td>
</tr>
<tr>
<td>Tyrosine 3-Monoxygenase/Tryptophan 5-Monoxygenase Activation Protein</td>
<td><em>Ywhaz</em></td>
<td>Mouse</td>
<td>FAM</td>
<td>Mm03950126_s1</td>
</tr>
</tbody>
</table>
Ten genes selected as potential endogenous controls when exposed for inorganic mercury (2.0 mg Hg/L), measured with FAM labeled probes. Selection of endogenous controls were determined with Ct value and normfinder. The criterion for selection was based on minimal fluctuation of Ct values between samples in order to assume being independent of Hg exposure.

**Table S7. Housekeeping genes selected for Hg exposure**

<table>
<thead>
<tr>
<th></th>
<th>Gapdh (Ct)</th>
<th>Ppia (Ct)</th>
<th>Geometric mean (Ct)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A.SW Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17,427</td>
<td>19,368</td>
<td>18,371</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0,1702</td>
<td>0,307</td>
<td>0,206</td>
</tr>
<tr>
<td><strong>A.SW Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16,061</td>
<td>18,893</td>
<td>17,419</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0,530</td>
<td>0,457</td>
<td>0,475</td>
</tr>
<tr>
<td><strong>B10.S Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>19,228</td>
<td>21,554</td>
<td>20,358</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0,338</td>
<td>0,283</td>
<td>0,299</td>
</tr>
<tr>
<td><strong>B10.S Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18,180</td>
<td>20,597</td>
<td>19,350</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0,426</td>
<td>0,405</td>
<td>0,414</td>
</tr>
</tbody>
</table>

*Gapdh* and *Ppia* were selected as housekeeping genes after evaluation of 10 potential endogenous controls in Supplemental Material, Table S6. Ct, geometric mean Ct and Standard Deviation of Ct values for each group of mice are presented. Ct variation for housekeeping genes and their geometric mean is below 1 Ct.
Table S8. Codons and amino acids of SNPs on *Pprc1*

<table>
<thead>
<tr>
<th>Class</th>
<th>ID (Ensembl)</th>
<th>Transcript codon/amino acid A</th>
<th>Transcript codon/amino acid C57BL/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP¹</td>
<td>rs30400427</td>
<td>GAA Glu</td>
<td>AAA Lys</td>
</tr>
<tr>
<td>SNP²</td>
<td>rs30815571</td>
<td>AAT Asn</td>
<td>GAT Asp</td>
</tr>
<tr>
<td>SNP³</td>
<td>rs30566249</td>
<td>TCA Ser</td>
<td>CCA Pro</td>
</tr>
<tr>
<td>SNP⁴</td>
<td>rs30507907</td>
<td>CCT Pro</td>
<td>CTT Leu</td>
</tr>
<tr>
<td>SNP⁵</td>
<td>rs30750332</td>
<td>GTT Val</td>
<td>ATT Lle</td>
</tr>
<tr>
<td>SNP⁶</td>
<td>rs30360955</td>
<td>TCT Ser</td>
<td>CCT Pro</td>
</tr>
<tr>
<td>SNP⁷</td>
<td>rs30352970</td>
<td>AGT Ser</td>
<td>GGT Gly</td>
</tr>
</tbody>
</table>

Seven SNPs on exon 5 on *Pprc1* between A/J and C57BL/6J. Accession numbers followed by transcript codons and amino acids of the seven non-synonymous SNPs (1-7) on *Pprc1* are presented. Bold character represents the SNP on each codon.
**Figure S1.** Conserved regions of SNPs 1-6

**SNP^1:** rs30400427, A.SW (GAA), B10.S (AAA)

**SNP^2:** rs30815571, A.SW (AAT), B10.S (GAT)

**SNP^3:** rs30507907, A.SW (CCT), B10.S (CTT)

**SNP^4:** rs30750332, A.SW (GTT), B10.S (ATT)

**SNP^5:** rs30360955, A.SW (TCT), B10.S (CCT)
Comparison of conserved regions on 15 mammals on SNPs\textsuperscript{1-6} according to Ensembl database. Selection of mammals is due to that Ensembl database have run a nucleotide alignment against them specifically. Accession number followed by codons of A.SW and B10.S are presented in which bold character represents the SNP.
Fifteen mammalian species were selected, in which Ensembl database have run a nucleotide alignment against on rs30352970 in *Pprcl*. Sequence data of all 15 mammals were obtained by using Ensembl database for retrieval of amino acid sequences. Conserved region on amino acids were performed by aligning the multiple sequences with the use of Clustal X (version 2.1).
**Figure S3.** Amino acid alignment of SNP7 in *Pprc1*

<table>
<thead>
<tr>
<th>Species</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gorilla</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Orangutan</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Human</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Vervet</td>
<td>YRSGLKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Olive</td>
<td>YGSGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Marmoset</td>
<td>YGGLKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Rabbit</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Cow</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Sheep</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Pig</td>
<td>YGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Dog</td>
<td>YPGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Cat</td>
<td>YPGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Horse</td>
<td>YPGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Mouse</td>
<td>YPGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
<tr>
<td>Rat</td>
<td>YPGPGKGPWPCHFFNSTVPPPPPAPASLGRAPQKMSRGTPTCPPENVLPSPAPLSLSLIGPGHAP</td>
</tr>
</tbody>
</table>

Comparison of conserved region on 36 mammals and three birds, of SNP7 (rs30352970) according to Ensembl database. Selection of animals is due to that Ensembl database have run a nucleotide alignment against them specifically. Accession number followed by codons of A.SW and B10.S are presented in which bold character represents the SNP.