

Microarray Analysis of Mercury-Induced Changes in Gene Expression in Human Liver Carcinoma (HepG₂) Cells: Importance in Immune Responses

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Abstract: Mercury is widely distributed in the biosphere, and its toxic effects have been associated with human death and several ailments that include cardiovascular diseases, anemia, kidney and liver damage, developmental abnormalities, neurobehavioral disorders, autoimmune diseases, and cancers in experimental animals. At the cellular level, mercury has been shown to interact with sulphhydryl groups of proteins and enzymes, to damage DNA, and to modulate cell cycle progression and/or apoptosis. However, the underlying molecular mechanisms of mercury toxicity remain to be elucidated. Our laboratory has demonstrated that mercury exposure induces cytotoxicity and apoptosis, modulates cell cycle, and transcriptionally activates specific stress genes in human liver carcinoma cells. The liver is one of the few organs capable of regeneration from injury. Dormant genes in the liver are therefore capable of reactivation. In this research, we hypothesize that mercury-induced hepatotoxicity is associated with the modulation of specific gene expressions in liver cells that can lead to several disease states involving immune system dysfunctions. In testing this hypothesis, we used an Affymetrix oligonucleotide microarray with probe sets complementary to more than 20,000 genes to determine whether patterns of gene expressions differ between controls and mercury (1-3µg/mL) treated cells. There was a clear separation in gene expression profiles between controls and mercury-treated cells. Hierarchical cluster analysis identified 2,211 target genes that were affected. One hundred and thirty-eight of these genes were up-regulated, among which forty three were significantly over-expressed ($p = 0.001$) with greater than a two-fold change, and ninety five genes were moderately over-expressed with an increase of more than one fold ($p = 0.004$). Two thousand and twenty-three genes were down-regulated with only forty five of them reaching a statistically significant decline at $p = 0.05$ according to the Welch's ANOVA/Welch's t-test. Further analyses of affected genes identified genes located on all human chromosomes except chromosome 22 with higher than normal effects on genes found on chromosomes 1-14, 17-20 (sex-determining region Y)-box18SRY, 21 (splicing factor, arginine/serine-rich 15 and ATP-binding), and X (including BCL6-co-repressor). These genes are categorized as control and regulatory genes for metabolic pathways involving the cell cycle (cyclin-dependent kinases), apoptosis, cytokine expression, Na⁺/K⁺ ATPase, stress responses, G-protein signal transduction, transcription factors, DNA repair as well as metal-regulatory transcription factor 1, MTF1 HGNC, chondroitin sulfate proteoglycan 5 (neuroglycan C), ATP-binding cassette, sub-family G (WHITE), cytochrome b-561 family protein, CDC-like kinase 1 (CLK1 HGNC) (protein tyrosine kinase STY), Na⁺/H⁺ exchanger regulatory factor (NHERF HGNC), potassium voltage-gated channel subfamily H member 2 (KCNH2), putative MAPK activating protein (PM20, PM21), *ras* homolog gene family, polymerase (DNA directed), δ regulatory subunit (50kDa), leptin receptor involved in hematopoietin/interferon-class (D200-domain) cytokine receptor activity and thymidine kinase 2, mitochondrial TK2 HGNC and related genes. Significant alterations in these specific genes provide new directions for deeper mechanistic investigations that would lead to a better understanding of the molecular basis of mercury-induced toxicity and human diseases that may result from disturbances in the immune system.

Keywords: Mercury, oligonucleotide microarray, gene expression profile, HepG₂ cells, immune responses.

Introduction

Mercury is a biohazardous metal found naturally in the environment in different chemical species. Sample analysis shows that an average 70 kg man has mercury deposit of about 13 mg in the skin, nails, hair, and kidneys [1]. In growing children mercury tends to have a pronounced neurotoxic effect in the central (cns) and peripheral (pns) nervous systems. To some groups of genetically prone individuals exposure to the metal leads to the development of immune-dysfunctions. In such people mercury is a potent dose-dependent immunostimulant that initiates a number of immuno-pathologic diseases with raised lymphocyte proliferation, increased blood immunoglobulin levels and absolute systemic hyper-reactivities. Toxicity associated with mercury arises through avid bonding with sulfhydryl (-SH) and to a lesser degree hydroxyl, carboxyl, and phosphoryl groups. These linkages modify signal transduction events in the body [2]. Mercury in particular binds to functional cysteine-rich sequences in the extracellular domains of both CD95 and the tumor necrosis factor receptor [TNF-R] through trimer formation. The CD95/TNF ligands are a family of proteins that belong to a homotrimeric group of molecules individually capable of binding to three other CD95/TNF receptors respectively to form clusters of the receptors' death domains. Trimerization is a step necessary for the correct delivery of death signals in the course of apoptosis or programmed cell death (PCD) [3-5]. Receptor trimerization induced by bivalent inorganic mercury Hg^{2+} for instance results in nonspecific, dysregulated signal transduction and disorders of cellular functions. Cellular dysfunctions may involve inactivation of enzymes, changes in protein conformations, inhibition of several transport processes that may disrupt permeability properties of cell membranes, the generation of free radicals and peroxides due to decline in activities of superoxide dismutase (SOD) and catalase to the involvement of glutathione peroxidase.

CD95/CD95L ligand apoptotic interactions can result in three physiological pathways that may be associated with: 1) regulation of T cells activities by their deletion at termination of an immune response, 2) destruction of targets such as virus-invaded cells or cancer cells by cytotoxic T cells and by natural killer cells, and 3) elimination of inflammatory cells at 'immune-privileged' sites, areas protected from injury through immune responses. Mutations occurring in the CD95 and/or CD95L culminate in the accumulation of peripheral lymphoid cells that leads to the induction of fatal autoimmune condition characterized by lymph nodes enlargement. CD95 (also called Fas or Apo1) and TNFR1 (or p55 or CD120a) are the familiar death receptors (DRs) that utilize activation induced cell deaths (AICD) in metabolic pathways. Other known death receptors are DR3 (Apo3), WSL-1, TRAMP, or LARD; DR4 and DR5, (also called Apo2), TRAIL-R2, TRICK 2, or KILLER [3]. Some tumors are known to express CD95L that interact with receptor to induce pathological state whereby suppression of immune surveillance by tumor-reactive immune cells occurs [5,

6]. The death effector domain is among a very widely distributed homophilic interaction of caspase recruitment domain (CARD) found in many caspases with large prodomains including caspases-2, -8, -9 and -10 [6-8].

The interaction of an adapter protein, Fas-associated death domain (FADD) to caspase-8 gives rise to oligomers that self cleave caspase-8 for activation. Caspase-8 is linked to the activation of downstream caspases such as caspase-9, the mammalian functional homolog of CED-3 that commits the cell to apoptosis. In some cell types TNF also induces apoptosis via TNFR1 [9, 10]. Trimerization occurring between TNF and its ligand, TNFR1 is known to stimulate production of NF- κ B and AP-1 transcription factors that lead to the induction of proinflammatory and immunomodulatory genes [7, 64]. Activated macrophages and T cells are the predominant cells that produce TNF during infections. These multi-pathways utilized by the Fas/TNF family signals are prime candidates for immunopathogenesis that give rise to the various life-threatening effects in several cell types [9-15]. For instance the triggering of Fas may induce PCD in activated T cells, but is costimulatory in resting T cells [13]. Molecules participating in Fas-signaling are also involved in signaling via other surface receptors. Sphingomyelinase is also known to be involved in signaling via several cytokine receptors [10-14]. Initiator caspases are the first to be activated and include caspase-2, 8, 9 and 10. These proteins cleave and activate the effector caspases (3, 6, 7), which cleave, degrade or activate other cellular proteins. Some caspases (1, 4, 5, 11, 12, 13, and 14) have a specialized role in inflammation and their activation leads to the processing of pro-inflammatory cytokines. Such array of possible pathways resulting from reactions of this family of proteins including CD95 and/or its ligand may explain why toxicity from mercury exposure is linked with several adverse health effects involving many body systems and invariably involves failure or enhanced activities of immune responses in man. For example mercury has been associated with cardiomyopathies and arrhythmias, other neurological problems such as tremors, insomnia, polyneuropathy, paresthesias, emotional lability, irritability, personality changes, headaches, weakness, blurred vision, dysarthria or speech impairment, slowed mental response, and unsteady gait [15-17]. The effects of mercury on human systems are numerous. A maculopapular rash, swollen nodes and painful extremities, peripheral neuropathy, hypertension, and renal tubular dysfunction develop in affected children [18]. However individual susceptibility to mercury is poorly understood.

Several proteins in the body including the caspases (cysteine proteases) carry -SH groups; -SH groups are components of several structural parts of cells, of enzymes and in most biochemical pathways, -SH groups participate in various metabolic paths occurring in the cell environ. In this respect importance is placed on chemokines and receptors now known to play important roles in inflammatory and immunologically mediated diseases. These cytokine peptides of 8-12 kDa bind to heparin and attract leucocyte subsets to specific sites in the course of the immune response. More than fifty of these chemokines have so far been isolated and classified

into four groups CXC, CC, C, and CX3C according to the arrangement of conserved cysteine residues. On interacting with their receptors they stimulate a large family of seven transmembrane spanning G proteins. These G protein-coupled receptors are differentially distributed among various cell types and send signals downstream in the membrane in a given cell. It is of note that genes encoding inflammatory chemokines are typically located on two major clusters on human chromosomes 4 (CXC) and 17 (CC), whereas genes for homeostatic chemokines are found in small clusters on chromosomes 1, 2, 5, 7, 9, 10, and 16. Homeostatic receptors include CXCR4, CXCR5, CCR4, CCR7 and CCR9. Inflammatory receptors include CXCR1, CXCR2, CXCR3, CCR1, CCR2, CCR3, CCR5 and CCR6. In most circumstances G protein activation end in cytoskeletal reorganization leading to alterations in motility as a result of *phospholipase C* and *rho* stimulation [10-14,19-21]. Under certain circumstances, however activation of protein tyrosine kinases results in cell activation and proliferation. Inducible and constitutive chemokines arising from inflammatory processes and components of bone marrow, thymus and secondary lymphoid organs respectively recruit leucocytes, dendritic cells and activated T cells and are involved in regulating physiological leucocyte trafficking, an important component of immune surveillance.

Chemokines are involved in several biochemical as well as immunological regulating activities including leucocyte circulation or its localization, retention, and positioning in tissues. Regulation of leucocyte recruitment is complex. Functionally they secrete and present chemokines in the target tissues as well as regulating the expression of chemokine receptors on leucocytes during differentiation and activation. They are also important component of polarized type 1 and type 2 T cell responses. Chemokines participate in several points in the course of T cell differentiation, directly affecting T cell activation as well as causing changes in potential migration of T cell by giving directions as to migration and which cell it interacts with. Chemokines affect the type and number of dendritic cells (DC) attracted to the site of inflammation and also influences how the DCs respond to T cell activation, processes leading to the selection of subtypes of these cells in the course of immune responses. For instance signals delivered to DCs via CCR5 can drive IL-12 secretion whereas signals from CCR2 inhibit IL12 and these cytokines will define the subsequent T cell differentiation. Inappropriate activation of these signaling proteins causes a cell to proliferate excessively [20-24]. In the end chemokine receptors expressed by effector T cells determine where they migrate and cause the final pathology. For instance increased interferon alpha (IFN- α) and IFN- ω are among the initial cytokines abnormally expressed in SLE patients [23-31].

In patients with spontaneously induced SLE high levels of IFN- α -inducible proteins (eg OAS1, OAS2, and OASL) and other proteins have been measured [25-38, 68]. IFN- α is associated with several immunomodulatory functions including its known antiviral action: it can stimulate immunoglobulin (Ig) synthesis in peripheral

blood mononuclear cells (PBMC) *in vitro* [39], maintains T cells alive [40], inhibits B cell receptor-mediated apoptosis [30], and is able to induce dendritic cell differentiation in SLE patients [37, 38]. IFN- α can also induce formation of anti-nuclear antibodies against native DNA that specify the SLE disease [39]. These conditions are found also in HgCl₂-treated rats [39-48]. For these reasons IFN- α has been observed to play a very significant role in the initial immunopathogenesis in SLE groups [26-38]. Similar multichannel pathways are also emerging from animal studies and patients suffering from diabetes and rheumatoid arthritis (RA) [49-57]. Confirmation from animal models of inflammatory arthritis and data from patients with rheumatoid arthritis reveal that several cytokines are produced in the course of the disease. Cytokines are considered to be factors that mediate communications between cells, and are involved in attracting inflammatory and immune cells into the joints where these cells emit products that are damaging to the tissues. Cytokines interact specifically with receptors on cell surfaces; stimulate pathways of signal transduction leading to high or low transcription. Two-signal transduction pathways that may be considered important in the rheumatoid synovium are the AP-1 and the NF- κ B pathways [11, 51]. NF- κ B seems to be particularly important in chronic inflammatory diseases, both in mediating IL-1 and TNF α production as well as in mediating their effects on target cells after binding to cell surface receptors. Stimulation of AP-1s and the NF- κ B signal transduction pathways lead to the release of collagenases and other enzymes, pro-inflammatory molecules, and more cytokines.

Further complications arise from the fact that several genes that encode for proteins in the intracellular signaling cascades that are activated by receptor tyrosines were initially isolated as oncogenes in cancer cells or tumor viruses [21, 25, 58, 59]. This finding implies that the origins of most diseases are linked via exposures to chemical and other environmental xenobiotics including mercury. Degree of toxicity depends on mercury species, dosage, individuality and the type of MHC association [10, 43-48]. Therefore genetic analyses and classification of mercury-susceptible MHC haplotypes may be an appropriate indicator of severity index to MeIA diseases; an index which can be expanded to show how these genes react to exposures to different types and levels of environmentally available xenobiotics in general. The immunological processes involved in immune enhancement and/or immune suppression are still not clearly defined. Till now genetic susceptibility to autoimmunity implicate high prevalence in individuals with the following combinations of genes in MHC-haplotype linkages: *FasL* (CD95/L), *Sap* (serum amyloid P-component), *Fc γ R2b* (Fc γ RIIB), *Cr2* (CD21/CD35) and *Ptprc* (CD45), whereas deficiency of *Fc γ R1g* (Fc γ -chain) results in resistance to autoimmunity. These genes are not by any means exhaustive [59]. Severity of autoimmune diseases seems to rely on the number as well as the type of linked MHC class genes on a chromosome. This is an indication that susceptibility and/or initiation factors operate via multiple pathways subjected to regulatory or checkpoints that finally give

rise to the pathological state as exemplified by SLE, IDDM and RA patients. In genetically predisposed patients the synthesis of autoantibodies and/or the generation of cellular attack of self antigens may follow different pathways. Such mechanisms are known to be influenced by genetic factors and contributions from ethnic and environmental backgrounds. The liver is the only organ that has the capacity to regenerate on demand by the body; thus dormant genes can be re-activated or induced by environmental factors. In this research the basic underlying hypothesis is that mercury-induced hepatotoxicity and related morbidities linked with immune problems is associated with modulation of specific gene expressions in liver cells. To test this hypothesis, Affymetrix Oligonucleotide microarray with probe sets complementary to more than 20,000 genes were used to determine differences, if any between patterns of gene expressions in controls and mercury (1-3 µg/mL)-treated cells.

Materials and Methods

Chemical Reagents and Growth medium

Mercury: Stock solution 10^4 µg/mL preserved in 10% HNO₃ CAS No. 7439-97-6 was purchased from EM Science (Gibbstown, New Jersey). Penicillin-Streptomycin, Lot No. 1085899, fetal bovine serum (FBS), and phosphate buffered saline (PBS) were purchased from Gibco-BRL Life Technologies (Grand Island, New York). Complete growth medium, DMEM, Trypsin EDTA and penicillin-streptomycin were purchased from American Type Culture Collection (ATCC), Manassas, VA. RNEasy, RNAlater and RNAlater RNA stabilization kits for RNA extraction were purchased from Qiagen, Inc. Streptavidin phycoerythrin (SAPE) protocol fluorescent labeling and hybridization control kit (containing 20 times reagent kit pre-mixed hybridization control transcripts for bioB, bioC, and bioD) with genes of the biotin synthesis pathway from the bacteria *E. coli*, and cre, (the recombinase gene from PI bacteriophage) were supplied by Affymetrix, Inc. (San Jose, California).

Cell Culture and Harvesting

Human transformed hepatoma, HepG₂ cell line, stored frozen under liquid nitrogen was thawed by gentle agitation of vials for 2 minutes in water bath at 37°C. The content of each vial was transferred to Petri dish and diluted with DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were incubated for a total of 48 hours at 37°C in a 5% CO₂ humidified environment. After the first 24 hours cells were washed in DMEM and further incubated for 24 hours to achieve approximately 95% confluence. This procedure was carried out for both control and test samples as outlined.

Mercury Exposure

To prepare cells for chemical exposure, a mercury stock solution of 10^4 µg/mL (in 10% HNO₃) was used to prepare standard solutions of 10, 20 and 30 µg/mL

concentrations in PBS. From these standard solutions equivalent volumes of 1, 2, and 3 mL were added to 19, 18, and 17 mL solutions of complete DMEM growth medium supplemented with 10% FBS and 1% penicillin-streptomycin to achieve final concentrations of 1, 2 and 3 µg/mL of mercury respectively. For the control samples similar dilutions of 1, 2, or 3 mL of PBS in 19, 18, and 17 mL of complete DMEM media were prepared. Cells were grown in petri dishes, washed with PBS, trypsinized with 10 mL (0.25%) (w/v) containing 0.03% EDTA (w/v) and harvested to 1×10^7 for total RNA extraction. Representative 1, 2, and 3 µg/mL mercury concentrations of HepG₂-treated and control cells were stabilized in 5 to 10 volumes of RNAlater RNA stabilization reagent for storage or for immediate RNA extraction as needed. RNAlater RNA stabilization kit (Qiagen Inc.) was recommended for RNA purification procedures to circumvent electrophoretic runs for monitoring extracted RNA purity.

Total RNA Extraction, Stabilization and Quantitation

Cells stored in RNAlater RNA stabilization reagent were thawed at 37°C in water bath with agitation and centrifuged at 300g for 5 min into pellet; the supernatant was discarded. All centrifugations were performed at 20-25°C in a standard micro centrifuge ensuring that the temperature did not cool below 20°C.

RNEasy kits (Qiagen) were used to isolate and purify RNA from the mercury treated and untreated HepG₂ cells. Samples were initially lysed and homogenized in the presence of a highly denaturing guanidine isothiocyanate (GITC)-containing buffer. This process immediately inactivates RNases to ensure isolation of intact RNA. Addition of equal volumes of ethanol provides appropriate binding conditions, the sample was then applied to an RNeasy mini column where the total RNA binds to the membrane and contaminations are efficiently washed away. High quality RNA was then eluted in 30 µL, or more, of RNase free deionized water. In such procedures all RNA molecules longer than 200 nucleotides are isolated providing an enriched RNA. The size distribution of purified RNA is comparable to that obtained by centrifugation through CsCl cushion, where small RNAs do not sediment efficiently. RNAlater RNA stabilization kit (Qiagen) was then added for RNA purification procedures that were monitored with the A₂₆₀/A₂₈₀ ratio. Concentration of extracted RNA was computed based on equivalency of 40 µg/mL of RNA per mL in RNase free deionized water taking into account the amount of RNase free deionized water used for the final elution (between 30-50 µL) dependent on amount of extract. Optical readings at A₂₆₀ and A₂₈₀ nm Absorbance (A) of RNA extracts were carried out using UV/VIS/NIR spectrophotometer Lambda 20 (Perkin Elmer). Purity of RNA was analyzed utilizing the RNAlater stabilization technique. Amounts of RNA extracted were calculated and accepted or disregarded for further RNA extraction dependent on sample A₂₆₀/A₂₈₀ absorbance ratio meeting the set criteria limits of 1.8 to 2.1 in the RNAlater stabilization procedure. RNA extractions were done for each concentration of mercury-treated and control cells prepared at concurrent times.

Hybridization of Biotin-labeled cRNA to Oligonucleotide Microarrays and Staining Procedures

Procedures for probe hybridization as laid out in the Affymetrix Technical Gene Chip manual, version 5.0 were followed. Hybridization control kit containing 20 times reagent kit with pre-mixed hybridization control transcripts bioB, bioC, and bioD was utilized as directed. For each target the scaling up volume of Midi array buffer mixtures for hybridization (final volume 200 μ L) were prepared as outlined and arrays were normalized to room temperature, hybridization cocktail heated to 99°C for 5 minutes and spun in microcentrifuge for another 5 minutes. Supernatant cocktail was used to fill the probe arrays as recommended by Affymetrix, Inc.

Probe arrays were placed in the rotisserie motor kept at 45°C and rotated at 60 rpm for 16 hours using the Affymetrix GeneChip Hybridization Oven 640 for processing. Samples were removed from the probe arrays and freshly filled with micro filtered stringent buffer. Hybridization was continued in Affymetrix GeneChip Fluidics station followed by probe staining procedures using stringent and non-stringent wash buffer in Affymetrix Standard Format (Euk-WS2) [60]. Probe arrays were processed using three-stain streptavidin phycoerythrin (SAPE) protocol. Triplicate RNA extracts from each of the three concentrations 1, 2, and 3 μ g/mL were used for hybridization procedures.

Microarray Image and Probe Array Scanning

Streptavidin-stained genes hybridized to u133 Affymetrix chips series were scanned for interrogation using Affymetrix Microarray Suite scanner engaging argon-ion laser equipped with a safety interlock system. Scanner was set at 2 X image scan, 3 μ m pixel values, at wavelength 570 nm for 50 μ m probe arrays with probe cells 24 μ m or less. For each gene, the relative expression in the exposed as compared to the control or baseline was determined for each cDNA. Internal controls employed during hybridization were kindly supplied by Affymetrix Inc. to normalize for differences in mRNA quality and efficiency of probe labeling. This procedure improves data quality used for downstream analysis.

Statistical Analysis

Fluorescence signals emitted by the probes were converted to signal log ratios (SLR) correlating with measure of the abundance of a transcript. Analyses were performed using Affymetrix expression Batch Query employing the Wilcoxon's Signed Rank (WSR) test for comparisons of test mercury-treated (1-3 μ g/mL concentrations) and control HepG₂ cells. Stat common pairs, the intersection of the probe pairs from the baseline and experiment that are used by the expression algorithm to make the change call were generated as signal log ratio (SLR) reflecting the change in the expression level of a transcript between a baseline noise (control) versus an experimental array. This change is put out as the log₂ ratio. A log₂ signal ratio of 1 is equal to a fold change of 2. SLR, the quantitative change in transcript abundance estimates the magnitude and

direction of change of a transcript of two arrays. From WSR test, a total of three, one-sided *p*-value was chosen to determine the change call between baseline and test sample. A combination of three of these values gives a final *p*-value provided in the data analysis output (.chp file). A range of *p*-values from 0.0 to 1.0 provides a measure of the likelihood of change and direction. The value closest to 0.5 signifies that no change is detected. Values close to 0.0 indicate likelihood for an increase in transcript expression level in the experiment array compared to the baseline, whereas values close to 1.0 indicate likelihood for a decrease in transcript expression level. *p*-value scales are used to generate discreet change calls using thresholds. The final change *p*-value is grouped by cutoff values referred to as gamma 1 (γ_1) and gamma 2 (γ_2) that provide boundaries for the change calls: I for increase; MI for marginal increase; D for decrease; MD for marginal decrease; NC for no change. Table 1 is a list of expression levels of genes located on human chromosomes as compared to the control sample when exposed to mercury at the concentrations indicated. Positive SLR indicates up-regulation; negative SLR indicates downregulation of the expressed genes. For detail mathematical computations of the Affymetrix Microarray suite refer to the User's Guide version 5.0 [60]. Corresponding probe pairs were compared between test and control sample utilizing the Welch's ANOVA/Welch's *t*-test. This procedure eliminates differences inherent in each probe binding coefficients and is, therefore more accurate than a single array analysis.

Results

Findings from this study indicate that mercury exposure has a significant effect on several genes expressed on most human chromosomes. There was a clear separation in gene expression profiles between controls and mercury-treated cells. Hierarchical cluster analysis identified 2,211 target genes that were affected. One hundred and thirty-eight of these genes were up-regulated, among which forty three were significantly over-expressed ($p = 0.001$) with greater than a two-fold change, and ninety-five genes were moderately over-expressed with an increase of more than one fold ($p = 0.004$) (Table 1). Two thousand and twenty-three genes were down-regulated with only forty five of them reaching a statistically significant decline at $p = 0.05$ according to the Welch's ANOVA/Welch's *t*-test (Table 1).

Affected genes were localized on all human chromosomes except chromosome 22; with higher activities on genes associated with chromosomes 1-10, 12, 14-18, 20 (sex-determining region Y), 21 (splicing factor and ATP-binding), X (including BCL-co-repressor). These genes include potassium voltage-gated channel-subfamily H member 2 (KCNH2), stress responses, G-protein signal transduction, putative MAPK activating protein (PM20, PM21), *ras* homolog gene family, cytokine receptor activity and polymerase (DNA directed), regulatory subunit (50kDa), leptin receptor involved in hematopoietin/interferon-class (D200-domain), and thymidine kinase 2, mitochondrial TK2 (HGNC) and related genes. It appears that the impact of mercury was concentration-dependent. Closely associated genes on a chromosome tend to be affected together (Table 1).

Table 1: Sample genes affected by mercury

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
209908_s_at	Transcript assignment(s)-GENSCAN0000002140 cdna: Genscan chromosome: NCBI35: 1:214900212:214908104:1 Alignment(s): Chr 1: 214907888-215003873(+) UCSC. Protein similarities: blast P09858- Transforming growth factor beta 2 precursor (TGF-beta 2); E value 0.0 Pfam- IPR001111 EMBL.EBI-Transforming growth factor beta (TGFb), N-terminal Pfam-IPR001839 EMBL.EBI -Transforming growth factor beta.	Align chr 1	5/32	4/16	1/2
206814_at	Nerve growth factor, beta polypeptide nerve growth factor, signal transducer activity /NGFB HGNC/—Cell-cell signaling development; 6112 energy reserve metabolism; 7166 cell surface receptor linked signal transduction; 7275 development; 4896 hematopoietin/ interferon-class (D200- domain) cytokine receptor activity.	1p13.1	1/2	2/4	3/8
208498_s_at	Amylase, alpha 1A; 1B; 1C (salivary); Amylase 2A, 2B(Pancreatic); AMY1A; AMY1B; AMY1C HGNC: AMY2A; AMY2B HGNC; 5975 carbohydrate metabolism; 7586 digestion; 5615 Extracellular space; 4556 alpha amylase activity; 5509 Calcium ion binding; 16798 hydrolase activity, acting on glycosyl bonds; 4556 alpha amylase activity; Pathway: starch and sucrose metabolism GENMAPP/KEGG.	1p21	5/32	7/128	4/16
211096_at	pre-B-cell leukemia transcription factor 2; PBX2 HGNC; 6355 regulation of transcription; 7387 anterior compartment specification; 7388 posterior compartment specification; 15977 carbon utilization by fixation of carbon dioxide; 5634 nucleus; 9573 ribulose bisphosphate carboxylase complex; 3700 transcription factor activity; 16984 ribulose-bisphosphate carboxylase activity.	1p21.3	5/32	4/16	0/1
213496_at	Plasticity related gene 1/LPPR4 HGNC; brain-specific phosphatidic acid phosphatase-like protein 1 [homo sapiens]	1p21.3	5/32	4/16	0/1
207255_at	Leptin receptor, LEPR HGNC; cytokine receptor gp130 cytokine binding-domains.	1p31	0/1	-1/-2	-1/-2
211703_s_at	Precursor beta- amyloid binding protein; BBP HGNC.	1p32.1	2/4	4/16	4/16
220611_at	Disabled homologue 1 (Drosophila); Development, neurogenesis; phosphotyrosine interaction domain. See above.	1p32-p31	2/4	4/16	4/16
205323_s_at	Metal-regulatory transcription factor 1; MTF1 HGNC; regulation of transcription from Pol 11 promoter; response to metal ion; transcription factor activity; transcription co-activator activity; zinc ion binding.	1p33	2/4	1/2	2/4

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
208628_s_at	Nuclease sensitive element binding protein 1; NSEP1 HGNC; 6355 regulation of transcription, DNA dependent; 6366 transcription from Pol 11 promoter; 9613 response to pest/ pathogen/ parasite; 5634 nucleus; 3677 DNA binding; 3690 double-stranded DNA binding; 3697 single-stranded DNA binding; 3700 transcription factor activity; 16564 transcriptional repressor activity.	1p34	3/8	1/2	1/2
219925_at	Zinc finger protein 258; ZN258 HGNC; development; membrane; DNA binding;	1p34.2	3/8	1/2	1/2
215268_at	KIAA 0754 protein; KIAA0754 HGNC; Protein similarities: blast ID XP 513340-Predicted: microfilament and actin filament cross-linker protein (Pan troglodytes); E value 0.0; blast ID BAC87042.1 unnamed protein product (Homo sapiens).	1p34.3	8/256	8/256	6/64
203503_s_at	Peroxisomal biogenesis factor 14; PEX14 HGNC/ peroxisomal anchor protein (Fragment); 5777 peroxisome; 5779 integral to peroxisomal membrane; 16020 membrane.	1p36.22	3/8	2/4	2/4
218731_s_at	von Willebrand factor A domain-related protein; WARP HGNC.	1p36.33	3/8	2/4	3/8
200006_at	7 Parkinson disease (autosomal recessive, early onset); PARK7 HGNC; Overlapping Transcripts: NM_007262 NCBI chromosome1: 7731338-7754882 (+) UCSC.	1p36.33-p36.12	3/8	2/4	3/8
214130_s_at	Phosphodiesterase 4D interacting protein (myomegadin); PDE4DIP HGNC; 6412 protein biosynthesis; 7010 cytoskeleton organization and biogenesis; 30036 actin cytoskeleton organization and biogenesis; 5622 intracellular; 5840 ribosome; 15629 actin cytoskeleton; 3735 structural constituent of ribosome; 3779 actin binding.	1q12	5/32	5/32	1/2
205076_s_at	Cisplatin resistance associated CRA HGNC.	1q12-q21	-1/-2	-1/-2	-1/-2
217048_at	SHC (Src homology 2 domain containing) transforming protein 1 SHC1 HGNC; 187 activation of MAPK; 1558 regulation of cell growth; 7176 regulation of epidermal growth factor receptor activity; 7242 intracellular signaling cascade; 8284 positive regulation of cell proliferation; 45840 positive regulation of mitosis; 5886 plasma membrane; 5068 transmembrane receptor protein tyrosine kinase adaptor protein activity. 5543 phospholipid binding; Pathway- Integrin-mediated_cell_adhesion GENMAPP/KEGG.	1q21	-1/-2	-1/-2	-1/-2

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
203411_s_at	Lamin A/C, Intermediate filament protein; muscle development.	1q21.2- q21.3	-6/-64	-4/-16	-4/-16
200652_at	Signal sequence receptor, beta (transloxon-associated protein beta); SSR2 HGNC; 6613 cotranslational membrane targeting; 5783 endoplasmic reticulum; 16021 integral membrane; 5048 signal sequence binding;	1q21-q23	-6/-64	-4/-16	-4/-16
204007_at	Fc fragment of IgG, low affinity IIIa, receptor for (CD16)/FCGR3A HGNC; low affinity immunoglobulin γ Fc region receptor III-B precursor (IgG Fc receptor III-1) Fc-γ RIII-β) Fc-γ RIIIB),(Fc-γRIII), (FcRIII) (CD16-B)(FcR-10).	1q23	-2/-4	-1/-2	-2/-4
201966_at	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase); NDUFS2 HGNC; 6118 Electron transport; 6120 mitochondrial electron transport, NADH to ubiquinone; 56243 membrane fraction; 5739 mitochondrion; 3954 NADH dehydrogenase) activity; 8137 NADH dehydrogenase activity (ubiquinone); 9055 electron carrier activity; 16491 oxidoreductase activity; 5489 complex 1_49Kd; electron transporter activity; 1.4e-205; Pathway: Electron_Transport_Chain GENMAPP/KEGG.	1q23	-2/-4	-1/-2	0/1
235401_s_at	Fc receptor homolog expressed in B cells; FREB HGNC; 4872-receptor activity.	1q23.1	-2/-4	-1/-2	-2/-4
218229_s_at	Pogo transposable element with KRAB domain/POGK HGNC; regulation of transcription, DNA-dependent; anti-apoptosis; development.	1q23.3	-2/4	-1/2	-3/-8
211188_at	CD84 antigen (leucocyte antigen) ; CD84 HGNC ; 6952 defence response ; 7156 homophilic cell adhesion ; 5887 integral to plasma membrane.	1q24	-5/-32	-6/-64	-4/-16
200844_s_at	Peroxiredoxin 6 PRDX6 HGNC;/antioxidant protein 2; non-selenium glutathione peroxidase.	1q24.2	-5/-32	-6/-64	-4/-16
206245_s_at	Influenza virus NS1A binding protein; IVNS1ABP HGNC; 6383 transcription from Pol III promoter; 8380RNA splicing; 9615 response to virus; 5667 transcription factor complex; 5681 spliceosome complex; 5515 protein binding.	1q25.1-q31.1	0/1	-1/-2	-3/-8

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
217602_at	Ubiquitin specific protease 39/USP39 HGNC; ubiquitin carboxy-terminal hydrolase family protein, ubiquitin protease, spliceosome assembly; mRNA processing; RNA splicing, cysteine-type endopeptidase activity; pre-mRNA splicing factor activity.	2p11.2	5/32	3/8	2/2
205871_at	Plasminogen-like; PLGL HGNC; plasmin activity not recorded.	2p11-q11	5/32	3/8	5/32
200671_s_at	Spectrin, beta, non-erythrocytic1/SPTBN1 HGNC; Duchenne muscular dystrophy protein, brain isoform (fragment); beta spectrin 2.	2p21	4/16	3/8	2/4
211615_s_at	Leucine-rich PPR-motif containing/LRPPRC HGNC; bicoid stability factor.	2p22.1	0/1	-2-4	0/1
219020_at	HS1-binding protein 3 /FLJ14249 HGNC; intracellular signaling cascade	2p24.2	0/1	-1/-2	-1/-2/
218495_at	Ribonuclease H1; RNASEH1 HGNC/ magnesium ion binding, RNA binding, endonuclease activity, ribonuclease H activity, hydrolase activity, maseH;nucleic acid binding	2p25	-1/-2	-4/-16	-2/-8
215074_at	Myosin 1B;MYO1B; 3774 motor activity; 3779 actin binding; 5516 calmodulin binding; 5524 ATP binding.	2q12-q34	5/32	1/2	4/16
220559_at	Engrailed homolog 1; 1501 skeletal development; 6355 regulation of transcription;, DNA-dependent; 9653 morphogenesis; 5634 nucleus; 3700 transcription factor activity; Protein domains- a.4.1 Homeodomain- All alpha proteins; DNA/RNA-binding 3-helical bundle; Homeodomain-like.	2q13-q21	6/64	2/4	4/16
215508_at	BUB budding uninhibited benzimidazole 1 homolog (yeast); BUB1 HGNC; 910 cytokinesis;6468 protein amino acid phosphorylation; 7049 cell cycle; 7067 mitosis; 7094 mitotic spindle checkpoint; 8283 cell proliferation; 776 kinetochore; 5634 nucleus; 5816 spindle pole body; 4674 protein serine/threonine kinase activity; 5524 ATP binding; 16740 transferase activity; Pathway: Cell_cycle_KEGG GenMAPP/KEGG.	2q14	6/64	0/1	0/1
210346_s_at	CDC-like kinase 1/CLK1 HGNC; regulation of cell cycle, cell proliferation; 4674 nucleus protein serine/threonine kinase activity; 4715 non-membrane spanning protein tyrosine kinase activity; 5524 ATP binding; 16740 transferase activity; 4672 pkinase;protein kinase activity; 3.5e-82. Pathway: phosphatidylinositol signaling system GENMAPP/KEGG.	2q33	0/1	-2/-4	2/4

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Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
202019_s_at	Lantibiotic synthetase component C-like/ LANCL1 HGNC	2q33-q35	-2/-4	-3/-8	-2/-4
210495_x_at	Fibronectin 1: FN1 HGNC; 6953 acute- phase response; 7155 cell adhesion; 9611 response to wounding; 16477 cell migration; 5576 extracellular; 5201 extracellular matrix structural constituent; 5518 collagen binding; 8201 heparin binding. Pathway: Inflammatory_Response_Pathway GENMAPP/KEGG.	2q34	-2/-4	-3/-8	-2/-4
215151_at	DOCK 10 HGNC; dedicator of cytokinesis 10; overlaps with ABO 14594 NCBI. Homo sapiens mRNA for KIAA0694 protein, partial cds.	2q36.3	7/128	4/16	7/128
216368_s_at	Collagen, type IV, alpha 3 (Goodpasture antigen/COL4A3 HGNC; induction of apoptosis, caspase activation, cell adhesion, cell surface receptor linked signal transduction, perception of sound, circulation, cell proliferation, negative regulation of cell proliferation and angiogenesis, integrin binding, metalloendopeptidase inhibitor activity.	2q36-q37	5/32	4/16	4/16
211452_x_at	Leucine rich repeat (FL11) interacting protein 1:LRRF1P1HGNC; 6357 regulation of transcription from Pol 11 promoter; 16481 negative regulation of transcription; 5634 nucleus; 5856 cytoskeleton; 3702 RNA polymerase 11 transcription factor activity; 3725 double-stranded RNA binding; 16564 transcriptional repressor activity.	2q37.3	5/32	4/16	4/16
215212_at	CDNA FLJ12091 fis, clone HEMBBID02582; Hs.549985 NCBI	Align: chr 3:180376149-180378570(+) UCSC	9/512	10/1024	5/32
207272_at	Zinc finger protein 80 (pT17); ZNF80 HGNC; 6350 Transcription; 6355 regulation of transcription, DNA-dependent; 5634 nucleus; 4700 transcription factor activity; 8270 Zinc ion binding.	3p12-qter	6/64	7/128	2/4
213024_at	TATA element modulatory factor 1; RMF1 HGNC; 6355 regulation of transcription, DNA-dependent; 6366 transcription from Pol 11 promoter; 3677 DNA binding; 3702 RNA polymerase II transcription factor activity; 3712 transcription cofactor activity.	3p21-p12	-1/-2	-1/-2	-1/-2

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

Probe Set ID (Affymetrix)	Gene Title (Target Description/Symbol)	Chromosome Location	1µg/mL Hg	2µg/mL Hg	3µg/mL Hg
			SLR Change/ Fold Change	SLR Change/ Fold Change	SLR Change/ Fold Change
207794_at	Chemokine (C-C) receptor 2; CCR2 HGNC; 6935 chemotaxis; 6954 inflammatory response; 6968 cellular defense response; 7186 G-protein coupled receptor protein signaling pathway; 7194 negative regulation of adenylate cyclase activity; 7204 cytosolic calcium ion concentration elevation; 7259 JAK-STAT cascade; 19735 antimicrobial humoral response (sensu Vertebrata); 5625 soluble fraction; 5887 integral to plasma membrane; 1584 rhodopsin-like receptor activity; 16493 C-C chemokine receptor activity. Pathway PCR _s _Class_A_Rhodopsin-like & Peptide_GPCRs GENMAPP/KEGG;	3p21	-1/-2	-1/-2	-1/-2
202009_at	PTK9L protein tyrosine kinase 9-like (A6-related protein); PRTK9L HGNC; 5622 intracellular 3779 actin binding; 5524 ATP binding; 16301 kinase activity.	3p21.1	-3/-8	-2/-4	-1/-2
201096_s_at	ADP-ribosylation factor 4/ARF4 <u>HGNC</u>	3p21.2-p21.1	-3/-8	-2/-4	-1/-2
39966_at	Chondroitin sulfate proteoglycan 5 (neuroglycan C)/CSPG5 HGNC; intracellular transport, neurogenesis; intracellular transport; Golgi vesicle membrane; membrane fraction; integral to plasma membrane; protein binding; growth factor activity.	3p21.3	5/32	4/16	4/16
242591_at	Heat shock regulated 1; XLHSRF-1HGNC; 7018 microtubule-based movement; 9612 response to mechanical stimulus; 30286 dynein complex; 3777 microtubule motor activity.	3p21.31	-1/-2	-1/-2	-3/-8
242243_at	TATA element modulatory factor 1; TMF1 HGNC; 6355 regulation of transcription, DNA-dependent; 6366 transcription from Pol 11 promoter; 3677 DNA binding; 3702 RNA polymerase II transcription factor activity; 3712 transcription cofactor activity.	3p21-p12	-1/-2	-1/-2	-1/-2
212308_at	Cytoplasmic linker associated protein 2; CLASP2 HGNC; 7076 mitotic chromosome condensation; 5488 binding. Protein Domain: Pfam-IPRO10705 mast, C-terminal EMBL-EBI.	3p23	6/64	3/8	1/2
201972_at	ATPase, H ⁺ transporting, lysosomal 70kDa, V1 subunit A ATPase; ATP6V1A HGNC; 6810 transport; 15986 ATP synthesis coupled proton transport; 15992 proton transport; 5887 integral to plasma membrane; 16469 proton-transporting two-sector ATPase complex; 5524 ATP binding; 16787 hydrolase activity; 16820 hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances; 46933 hydrogen-transporting ATP synthase activity, rotational mechanism; 46961; hydrogen-transporting ATPase, rotational mechanism. Pathways: oxidative phosphorylation; ATP synthesis; Photosynthesis; Flagella assembly; Type 111 secretion—GENMAPP/KEGG.	3q13.31	-2/-4	-3/-8	-3/-8

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
216752_at	Phosphoinositol-3-kinase, regulatory subunit 4, p150; PIK3R4 HGNC; 6468 protein amino acid phosphorylation; 7076 mitotic chromosome condensation; 7126 meiosis; 4674 protein serine/threonine kinase activity; 5488 binding; 5524 ATP binding; 16740 transferase activity. E value: 0.0	3q21.3	4/16	7/128	5/32
210703_at HG U133	Human; Position: 145481606-145485595 (+) UCSC. PRO2259 mRNA, complete cds	Align 3q24	4/16	0/1	3/8
207396_s_at	ALG3 HGNC Asparagine-linked glycosylation 3 homolog (yeast, α-1,3-mannosyltransferase); lethal (2) neighbor of tid; 6486 protein amino acid glycosylation; 5783 endoplasmic reticulum, 30176 integral to endoplasmic reticulum membrane; 30 mannosyltransferase activity; 16757 glycosyl transferase activity.	3q27.3	1/2	0/1	1/2
207009_at	Paired-like homeobox 2b; PHOX2B HGNC; 6355 regulation of transcription, DNA dependent; 7275 development; 7399 neurogenesis; 5634 nucleus; 3700 transcription factor activity; 3712 transcription cofactor activity.	4p12	6/64	4/16	5/32
222336_at	Hypothetical protein LOC 201895. Transcription Assignments- cDNA Genescan: Chromosome: NCBI 35:4:39380614:39429366:-1.	4p14	5/32	5/32	5/32
213980_s_at	C-terminal binding protein 1; CTBP1 HGNC; 6468 protein amino acid phosphorylation; 6564 L-serine biosynthesis; 8285 negative regulation of cell proliferation; 19079 viral genome replication; 5634 nucleus; 5794 Golgi apparatus; 8022 protein C-terminus binding; 16491 oxidoreductase activity; 16616 oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as receptor;	4p16	6/64	5/32	2/4
211062_s_at	Carboxypeptidase Z; CPZ HGNC/ transmembrane receptor activity; 3.4.17.10; carboxypeptidase E activity2.26e-134.	4p16.1	6/64	5/32	2/4
206639_x_at	Histatin 1; HTN1 HGNC; 1503 ossification; 6805 xenobiotic metabolism; 42742 defense response to bacteria; 50832 defense response to fungi; 5576 Extracellular region.	4q13	3/8	3/8	3/8
219667_s_at	B-cell scaffold protein with ankyrin repeats 1; BANK1 HGNC/	4q24	3/8	6/64	6/64

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Table 1 contd.: Sample genes affected by mercury.

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216720_at	Cytochrome P ₄₅₀ , family2, subfamily U, polypeptide 1 CYP2U1 HGNC; 16712 oxidoreductase activity, acting on paired donors, with incorporation of or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen.	4q25	5/32	6/64	4/16
207928_s_at	Glycine receptor, alpha 3; GLRA3 HGNC; GABA-A receptor activity; ion channel activity; extracellular ligand-gated ion channel activity, glycine binding (receptor, alpha 3 subunit), glutamate-gated chloride channel activity; neurotransmitter receptor activity; synaptic transmission.	4q33-q34	6/64	10/1024	8/256
213963_s_at	Sin3-associated polypeptide, 30kDa; histone deacetylase complex; transcription corepressor activity.	4q34.1	4/16	3/8	1/2
215378_at	Ankyrin repeat and KH domain containing 1; ANKHD1 HGNC; nucleic acid binding; cell cycle inhibitor p16ink4A; immunoglobulin heavy chain variable domain, VH; transcription factor NusA, receptor, different EGF domains; Class II MHC alpha chain, C-terminal domain; Silencer of death domains, Sodd (Bag 4); Staphylokinase.	5q31.3	2/4	2/4	2/4
207182_at	Gamma-aminobutyric acid (GABA) A receptor, alpha 6 (GABRA6 HGNC)	5q34	3/8	8/256	3/8
203812_at	Slit homolog 3 (Drosophila; SLIT3 HGNC); 7399 neurogenesis, 5615 extracellular space; 5509 calcium ion binding, 5515 protein binding.	5q35	0/1	-1/-2	-1/-2
207471_at	Representative Public ID AF 118086 NCBI; Grade A annotation; Transcript Assignment(s): AF118086 NCBI Homo sapiens PRO1992 mRNA, cds. 11/11 Chr6: 88330288-88332009(+) UCSC; unknown protein.	Align chr 6	9/512	7/128	5/32
202401_s_at	Serum response factor (c-fos serum response element-binding transcription factor)/SRF HGNC	6p21.1	-1/-2	-1/-2	0/1
209823-x_at	Overlaps with Major histocompatibility complex, class II, DQ β1 (HLA-DQβ1 HGNC); 6955 immune response; 19884 antigen presentation, exogenous antigen via MHC class II; 16021 integral to membrane. 45012 MHC class II receptor activity.	6p21.3	-1/-2	-1/-2	0/1
203290_at	HLA-DQA1 HGNC; major histocompatibility complex, class II,DQ alpha 1 6955 immune response; 19884 antigen presentation, exogenous antigen via MHC class II; 5887 integral to plasma membrane; 16021 integral to membrane; 45012 MHC class II receptor activity.	6p21.3	-1/-2	-1/-2	0/1
210208_x_at	HLA-B associated transcript 3; BAT3 HGNC.	6p21.3	-1/-2	-1/-2	0/1

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Table 1 contd.: Sample genes affected by mercury.

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214509_at	Histone 1, H31. Transcripts alignment(s)- NM_003533NCBI-Homo sapiens histone 1, H3i (HIST1 H31), mRNA 11/11	6p22-p21.3	4/16	0/1	0/1
207156_at	Alignments: chr6: 27947602-27948078(-) UCSC. H2ag histone 1/HIST1H2AG HGNC; DNA binding; 1.2e-53; H2A histone family,	6p22.1	3/8	5/32	7/128
200890-s-at	Signal signal receptor, alpha (translocon-associated protein alpha)/SSR1 <u>HGNC</u>	6p24.3	0/1	0/1	0/1
214790_at	SUMO1/sentrin specific protease 6 SENP6 HGNC; 6508 proteolysis and peptidolysis; 6512 ubiquitin cycle; 8234 cysteine-type peptidase activity.	6q13-q14.3	4/16	5/32	3/8
207054_at	Interphotoreceptor Matrix Proteoglycan 1; IMPG1 HGNC; 7601 visual perception; 5578 Extracellular matrix (sensu metazoa); 4872 Receptor activity; 5201 Extracellular matrix structural constituent.	6q14.2-q15	6/64	4/16	8/256
211387_x_at	RNA guanylyltransferase and 5'-phosphatase; RNGTT HGNC; mRNA capping, protein amino acid dephosphorylation, nucleic acid binding, transferase activity, hydrolase activity. Tyrosine specific protein phosphatase and dual specificity protein phosphatase.	6q16	3/8	3/8	-1/-2
205029_s_at	Fatty acid binding protein 7, brain; FABP7 HGNC; 6631 FA metabolism; 6810 transport; 7399 neurogenesis; 8285 negative regulation of cell proliferation; 5215 transporter activity; 5478 Intracellular transporter activity; 8289 lipid binding.	6q22-q23	7/128	5/32	8/256
217829_s_at	Peptidylprolyl isomerase A (cyclophilin A); protein folding; antimicrobial humoral response (sensu Vertebrata); regulation of viral genome replication; chaperone activity; peptidyl-prolyl cis-trans isomerase activity; protein transporter activity.	7p13-p11.2	4/16	0/1	3/8
201293_x_at	Peptidylprolyl isomerase A (cyclophilin A); PPIA HGNC; 6457 protein folding; 19735 antimicrobial humoral response (sensu Vertebrata); 45069 regulation of viral genome replication; 3754 chaperone activity; 3755 peptidyl-prolyl cis-trans isomerase activity; 8565 protein transporter activity; 16853 isomerase activity. <i>Alignment with chromosomes 1-3, 5-21, and X.</i>	7p13-p11.2	4/16	0/1	3/8
208641_s_at	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1); RAC1 HGNC; 6928 cell motility; 6954 inflammatory response; 7155 cell adhesion; 7264 small GTPase mediated signal transduction; 9653 morphogenesis; 3924 GTPase activity; 5525 GTP binding; 3925 ras; small monomeric GTPase activity; 3e-92 extended.; 2.5e-84 extended.	7p22	7/128	4/16	2/4

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

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206202_at	Mesenchyme homeobox 2 (growth arrest specific homeobox); MEOX2 HGNC; 6355 regulation of transcription, DNA-dependent; 7275 development; 8015 circulation; 5634 nucleus; 3700 transcription factor activity.	7p22.1-p21.3	7/128	4/16	2/4
207166_at	Gamma transducing activity polypeptide 1 guanine nucleotide binding protein (G protein); GNGT1 HGNC/G-gamma; heterotrimeric G-protein GTPase activity; 2.8e-26; gamma transducing activity polypeptide 1; photoreceptor transduction gamma subunit (fragment).	7q21.3	5/32	7/128	2/4
206552_s_at	Tachykinin, precursor 1(substance K, substance P, neurokinin 1 & 2, neuromedin L, neurokinin α , neuropeptide K, neuropeptide γ /TAC1 HGNC; 7217tachykinin signaling pathway; 7218 neuropeptide signaling pathway; 7267 cell-cell signaling; 7268 synaptic transmission; 7320 insemination; 9582 detection of abiotic stimulus; 5102 receptor binding; 5184 neuropeptide hormone activity.	7q21-q22	5/32	0/1	4/16
215516_at	Laminin, beta 4 LAMB4 HGNC. 5578 Extracellular matrix (sensu Metazoa); 5198 Structural molecule activity. Protein Domain-Pfam IPP008211-Laminin, N-terminal EMBL-EBI; Pfam IPR002049-Laminin-type EGF-like domain EMBL-EBI.	7q22-q31.2	5/32	6/64	2/4
209466_x_at	Pleiotrophin (heparin binding growth factor 8, neurite growth promoting factor 1); neurotrophic factor/PTN HGNC; osteoblastic specific factor-1, OSF-1.	7q33-q34	1/2	0/1	0/1
207166_at	Potassium voltage-gated channel, subfamily H(eag-related, member 2/KCNH2 HGNC;	7q35-q36	5/32	7/128	2/4
205262_at	6812 cation transport; 160 two component signal transduction system (phosphorylase) 6813 potassium ion transport; 6936 muscle contraction, 7605 perception of sound, 8016 regulation of heart rate; 6355 PAC; regulation of transcription, DNA dependent; 912e-09; 5624 membrane fraction; 8076 voltage-gated potassium channel complex; 16021 integral membrane; 155 two-component sensor molecule activity; 5251 delayed rectifier potassium channel activity; 5216 ion_trans; ion channel activity; 6.1e-31.	7q35-q36	5/32	7/128	2/4
209441_at	Rho-related BTB domain containing 2/RHO BTB2HGNC; ras, small monomeric GTPase activity; 1.3e-16; 5515 protein binding; 5525 GTP binding	8p21.2	5/32	3/8	3/8

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Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
207987_s_at	Gonadotropin-releasing hormone 1(Leutenizing-releasing hormone; GNR1 HGNC;signal transduction, cell-cell signaling, development, negative regulation of cell proliferation; leutenizing; GnRH.	8p21-p11.2	5/32	2/4	1/2
219312_s_at	Zinc finger and BTB domain containing 10 ZBTB10 HGNC; 6350 transcription; 6355 regulation of transcription, DNA-dependent; 5634 nucleus; 3677 DNA binding; 5515 protein binding; 8270 Zinc ion binding. E value: 0.0	8q13-q21.1	3/8	2/4	2/4
204865_at	Carbonic anhydrase III, muscle specific; CA3 HGNC; 6730 one carbon compound metabolism; 5737 cytoplasm; 4089 carbonic anhydrase activity; 8270 Zinc ion binding; 16829 Lyase activity; Pathway: Nitrogen metabolism GENMAPP/KEGG.	8q13-q22	6/64	3/8	4/16
209066_x_at	Ubiquinol-cytochrome c reductase binding protein; UQCRB HGNC; 8121 UCR_14kD; ubiquinol-cytochrome-c reductase activity;4.7e-56. Pathway: Oxidative phosphorylation;	8q22	6/64	3/8	4/16
205528_s_at	Electron Transport Chain GENMAPP/KEG. Runt-related transcription factor 1;translocated to, 1(cyclin D-related); RUNX 1TI HGNC; 6091 generation of precursor metabolites and energy; 6350 transcription; 6355 regulation of transcription, DNA-dependent; 5634 nucleus; 3700 transcription factor activity; Protein Domain: Pfam: IPR 000040 EMB-EBI Acute myeloid leukemia 1 protein (AML1)/RUNT; IPR 002893 EMB-EBI Zn-finger, MYND type.	8q22	5/32	1/2	3/8
200640_at	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide/YWHAZ HGNC; 19904 protein domain specific binding; 14-3-3 protein/cytosolic phospholipase A2 (Fragmnet); alignment(s) chr 10, 15, 2, 6, 8,9 and x.	8q23.1	-4/-16	-5/-32	-4/-16
244089_at	v-myc myelocytomatosis viral oncogen homolog(avian); MYC HGNC; 7050 cell cycle arrest; 6879 iron ion homeostasis, 8283 cell proliferation; 5634 nucleus; 6357 regulation of transcription from Pol II promoter; 3700 transcription factor activity; Pathway: Apoptosis GENMAPP/KEGG; Wnt_signaling GENMAPP/KEGG.	8q24.12-q24.13	1/2	0/1	1/2
212654_at	Tropomyosin 2 (beta)/TPM2 HGNC; muscle development; cytoskeleton; muscle thin filament tropomyosin.	9p13.2-p13.1	6/64	2/4	3/8
207932_at	Interferon, alpha 8 IFNA 8HGNC; 6952 Defense response; 5576 extracellular region; 5126 hematopoietin/interferon-class (D200-domain) cytokine receptor binding. E value 1.0E-102.	9p22	5/32	4/16	3/8

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

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209819_at	Hyaluronan binding protein 4 HABP4 HGNC Annotation transcript cluster (# of matching probes) NM_014282(11), u77327 (11); Transcript Annotations- u77327 NCBI; NM 014282- Human Ki-1/57 intracellular antigen mRNA, partial cds. 11/11 A. Homo sapiens hyaluronan binding protein 4 (HABP4) 11/11	9q22.3-q31	4/16	4/16	3/8
202491_s_at	Kinase complex-associated protein inhibitor kappa light polypeptide gene enhancer in B-cells; IKBKAP HGNC; 6461 protein complex assembly; 6468 protein amino acid phosphorylation; 6955 immune response; 4871 signal transducer activity; 8607 phosphorylase kinase regulator activity; 16301 kinase activity.	9q31	-2/-4	0/1	-4/-16
211402_s_at	Nuclear receptor subfamily 6, group A, member 1, NR6A1 HGNC; nuclear hormone receptor; transcription factor activity; regulation of transcription, spermatogenesis, cell proliferation, steroid hormone receptor activity, Err2; Orphan nuclear receptor NR5a2 (LRH-1).	9q33-q34.1	1/2	0/1	1/2
218813_s_at	SHE-domain GRB2-like endophilin B2/SH3GLB2 HGNC.	9q34	4/16	2/4	4/16
222241_at	chromosome 9 open reading frame 28; C9orf28 HGNC;(Full length) Hs.438972 NCBI; LocusLink 89853 Entrez gene .	9q34	4/16	2/4	4/16
217709_at	N-myristoyltransferase 2 NMT2 HGNC; EC 2.3.1.97; 6499 N-terminal protein myristylation; 9249 protein lipylation; 4379 glycolipid N-tetradecanoyltransferase activity; 8415 acyltransferase activity; 16740 transferase activity.	10p13	8/256	2/4	5/32
219433_at	5-OHtryptamine (serotonin) receptor 7 (adenylate-cyclase- coupled): G-protein signaling, coupled to cyclic nucleotide second messenger; synaptic transmission; circadian rhythm; circulation; Cellular component: integral to plasma membrane; rhodopsin-like receptor activity; melanocortin receptor activity; serotonin receptor activity.	10q21-q24	6/64	2/4	1/2
216098_s_at	5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled) serotonin-7 receptor pseudogene /HTR7, LOC 93164 HGNC; 7187 G-protein signaling, coupled to cyclic nucleotide second messenger; 7268 synaptic transmission; 7623 circadian rhythm; 8015 circulation; 5887 integral to plasma membrane; 1584 rhodopsin-like receptor activity; 4993 serotonin receptor activity; 4977melanocortin receptor activity. Pathway: GPCRDB_Class_A_Rhodopsin-like; monoamine_GPCRs GENMAPP/KEGG	10q21-q24	8/256	6/64	4/16

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
210110_x_at	Heterogenous nuclear ribonucleoprotein H3 (2H9) HNRPH3 HGNC; 6397 mRNA processing; 5634 nucleus; 30529 ribonucleoprotein complex; 3723 RNA binding; 3676 nucleic acid binding; 8e-12 extended.	10q22	8/256	6/64	4/16
202361_at	SEC24 related gene family, member C (S. cerevisiae); SEC24C HGNC; 6886 intracellular protein transport; 6888 EDR to Golgi transport; 5783 endoplasmic reticulum; 5794 Golgi apparatus; 30127 COP11 vesicle coat; 3779 actin binding; protein binding; 8565 protein transporter activity.	10q22.3	8/256	6/64	4/16
207661_s_at	SH3 multiple domains 1; SH3MD1 HGNC;	10q25.1	3/8	5/32	2/4
205493_s_at	Dihydropyrimidinase-like 4; DPYSL4 HGNC; 7399 neurogenesis; methylenetetrahydrofolate dehydrogenase/ cyclohydrolase; 16787 hydrolase activity.	10q26	8/256	6/64	8/256
201458_s_at	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)/BUB3 HGNC; 7067 mitosis; 7094 mitotic spindle checkpoint; 8283 cell proliferation; 776 kinetochore; 5634 nucleus. Pathway: Cell_cycle GENMAPP/KEGG.	10q26	-1/-2	-3/-8	-3/-8
208229_at	Fibroblast growth factor receptor (FGF) 2 (bacterial expressed kinase, keratinocyte growth factor receptor; craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)/FGFR2 HGNC; 6468 protein amino acid phosphorylation; 16021 integral to membrane; 4713 protein-tyrosine kinase activity; 4872 receptor activity; 5007 FGF receptor activity; 5524 ATP binding; 16740 transferase activity.	10q26	3/8	0/1	0/1
221950_at	Empty spiracles homolog 2(Drosophila) EMX2 HGNC; Homeobox protein EMX1; transcription factor activity; regulation of transcription, DNA-dependent.	10q26.1	5/32	8/256	4/16
221127_s_at	Regulated in glioma; RIG HGNC; aligns with 11p15.3	11p15.1	5/32	4/16	2/4
208607_s_at	Serum amyloid A2; SAA2 HGNC; 6953 acute phase response; 6954 inflammatory response; 5576 extracellular; 5319 lipid transporter activity; 3794 SAA_proteins; acute phase response protein activity; 1.3e-79. Serum amyloid A-2 protein precursor [contains: Amyloid protein A (Amyloid fibril protein AA)].	11p15.1-p14	5/32	4/16	2/4

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID</i> (Affymetrix)	<i>Gene Title</i> (Target Description/Symbol)	<i>Chromosome</i> <i>Location</i>	<i>1µg/mL Hg</i> <i>SLR</i> <i>Change/</i> <i>Fold</i> <i>Change</i>	<i>2µg/mL Hg</i> <i>SLR</i> <i>Change/</i> <i>Fold</i> <i>Change</i>	<i>3µg/mL Hg</i> <i>SLR</i> <i>Change/</i> <i>Fold</i> <i>Change</i>
200023_s_at	47kDa eukaryotic translation initiation factor 3, subunit 5 epsilon; EIF3S5 HGNC; 6412 protein biosynthesis; 6446 regulation of translational initiation; 3743 translational initiation factor activity. Pathway: Translational_Factors GENMAPP/KEGG.	11p15.4	-3/-8	-6/-64	-6/-64
210538_s_at	Baculoviral IAP repeat-containing 3, BIRC3 HGNC; 6916 anti apoptosis; 7166 cell surface linked signal transduction; 16567 protein ubiquitination; 42981 regulation of apoptosis; 151 ubiquitin ligase complex; 4842 ubiquitin-protein ligase activity; 5515 protein binding; 8270 zinc ion binding; Pathway: Apoptosis GENMAPP/KEGG	11q22	6/64	6/64	4/16
215355_at	POU domain, class 2, transcription factor 3/POU2F3 HGNC; regulation of transcription, DNA-dependent	11q23.3	7/128	5/32	4/16
220611_at	Male sterility containing 1 MLSTD1 HGNC	12p11.23	2/4	4/16	4/16
220615_s_at	Male sterity domain; MLSTD HGNC/ PGDH Human-EC: 1.1.1.141:15-Hydroxyprostaglandin Dehydrogenase [NAD (+)].	12p11.23	0/1	-3/-8	-1/-2
213764_s_at	Microfibrillar associated protein 5; MFAP5 HGNC; 1527 microbril; 5578 extracellular matrix (sensu Metazoa); 5615 extracellular space; 5201 extracellular matrix structural constituent.	12p13.1-p12.3	9/512	6/64	5/32
201604_s_at	Protein phosphatase 1, regulatory (inhibitor) subunit 12A/PPP1R12A HGNC; myosine phosphatase, target subunit 1 [Homo sapiens]	12q15-q21	2/4	1/2	1/2
208752__x_at	Nucleosome assembly protein-1-like 1/NAP1L1 HGNC; 6260 DNA replication; 6334 nucleosome assembly; 8284 positive regulation of cell proliferation; 5634 nucleus; 5678 chromatin assembly complex; 3677 DNA binding.	12q21.1	1/2	1/2	0/1
216466_at	Neuron navigator 3; nucleotide binding; pore membrane and/or filament interacting like protein 1; steerin 3 [homo sapiens]; Calponin-like actin-binding; rhodopsin-like GPCR superfamily ATPase	12q21.2	7/128	2/4	4/16
204840_s_at	Early Endosome Antigen 1, 162kD; EEA1 HGNC; 6906 vesicle fusion; 16189 synaptic vesicle to Endosomal fusion; 45022 Early Endosome to late endosome transport; 5624 membrane fraction; 5634 nucleus 5769 Early Endosome; 5829 cytosol; 19897 Extrinsic to plasma membrane; 3676 nucleic acid binding; 5516 Calmodulin binding; 5545 phosphatidylinositol binding; 8270 Zinc ion binding; 30742 GTP-dependent protein binding; 42803 protein homodimerization activity	12q22	2/4	5/32	2/4

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
201523_x_at	Ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast) HGNC ; 6282 regulation of DNA repair; 6464 protein modification; 6508 proteolysis and peptidolysis; 4840 ubiquitin conjugating enzyme activity; 4842 ubiquitin-protein ligase activity. Pathway: Ubiquitin mediated proteolysis GENMAPP/KEGG.	12q22	-3/-8	0/1	-2/-4
210515_at	Transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor transcription factor 1; RNA polymerase 11 transcription factor activity.	12q24.2	4/16	0/1	1/2
207956_x_at	Androgen-induced proliferation inhibitor; APRIN HGNC ; androgen-induced prostate proliferative shutoff associated protein; 6355 regulation of transcription, DNA-dependent 8283 cell proliferation; 8285 negative regulation of cell proliferation; 5634 nucleus; 3677 DNA binding; 5524 ATP binding.	13q12.13	-3/-8	-1/-2	-3/-8
204190_at	Chromosome 13 open reading frame 22; C13orf22 HGNC / 4197 cysteine-type endopeptidase activity; 4221 ubiquitin thioesterase activity;	13q12-q14	2/4	2/4	2/4
219471_at	Chromosome 13 open reading frame 18; C13orf18 HGNC ; (Full length-UniGene ID Build 170 () Hs.413071 NCBI.	13q14.11	2/4	2/4	2/4
211119_at	Estrogen receptor 2 (ER beta); regulation of transcription, DNA-dependent; signal transduction; cell-cell signaling; negative regulation of cell growth;steroid-binding; infertility. Ribosomal protein S29; RPS29 HGNC ; 3735 Ribosomal_S14; structural constituent of ribosome; 2.7e-13 extended.	14q	8/256	5/32	8/256
u133A 201094_at	Zinc finger and BTB domain containing 1; ZBTB1 HGNC /DNA binding; protein binding, zinc ion binding.	14q23.3	5/32	2/4	5/32
205092_x_at	Zinc finger and BTB domain containing 1; DNA-binding; protein binding; zinc ion binding; regulation of transcription, DNA-dependent.	14q23.3	5/32	2/4	5/32
220726_at	Chromosome 14 open reading frame 157/C14orf157 HGNC .	14q32.33	11/2048	11/2048	8/256
220726_at	Chromosome 14 open reading frame 157/C14orf157 HGNC . Representative Public ID- NM_024100 NCBI. Target transcription: gb: NM_025100.1/DEF= Homo sapiens hypothetical protein FLJ 12294/PROD= hypothetical protein etc.	14q32.33	11/2048	11/2048	8/256

SLR-- signal log ratios

Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
216892_at	Immunoglobulin heavy locus/IGH@ HGNC; IGHV3 protein (Fragment); CDNA FLJ25997 fis, clone DMC06923, highly similar to Ig gamma-1chain EMBL-EBI C region.	14q32.33	2/4	1/2	2/4
214328_s_at	Heat shock 90kDa protein 1, alpha; 6467 protein folding; 6839 mitochondrial transport; 7165 signal transduction; 42026 protein refolding; 45429 positive regulation of nitric oxide biosynthesis; 5829 cytosol; 166 nucleotide binding; 3754 chaperone activity; 3773 heat shock protein activity; 5515 protein binding; 5524 ATP binding; 30235 nitric oxide synthase regulator activity; 30911 TPR domain binding; 42803 protein homodimerization activity.	14q32.33	2/4	1/2	2/4
219196_at	Secretogranin III SCG3 HGNC; Transcript alignment(s)- NM_013243 NCBI-Homo sapiens secretogranin III (SCGS), mRNA.	15q21	9/512	8/256	9/512
209830_s_at	Solute carrier family 9(sodium/hydrogen) exchanger, isoform 3 regulator 2/SLC9A3R2 HGNC; 6461 protein complex assembly; 7242 intracellular signaling cascade; 5634 nucleus; 5886 plasma membrane; 5515 protein binding; blast- embi CAA90511.1/tyrosine kinase activating protein 1 (TKA-1) Homo Sapiens.	16p13.3	7/128	6/64	4/16
207756_at	Transcript Assignment(s) AKO 24372 NCBI- Homo sapiens cDNA FLJ14310fis, clone PLACE 3000271. Alignment(s) -chr16: 45516108-45519963(+) UCSC. Protein Similarities: blast P39189-Alu subfamily SB sequence contamination warning entry; blast AAG23169- HC6 (Homo sapiens)	Align Chr 16	7/128	3/8	7/128
216623_x_a	Trinucleotide repeat containing 9; TNRC9 HGNC; 785 chromatin; 5634 nucleus; 3677 DNA binding; blast-trinucleotide repeat containing 9 (Mus masculine).	16q12.1	8/256	6/64	9/512
211456_x_at	Metallothionein 1H; 46872 metal ion binding.	16q13	7/128	6/64	4/16
220575_at	Hypothetical protein FLJ11800 HGNC; alignment Ch 17 p11.2 / q23.1	Alignment Ch 17 p11.2 / q23.1	4/16	3/8	4/16
207704_s_at	Growth arrest-specific 7/GAS HGNC; 7050 cell cycle arrest; 7275 development; 7399 neurogenesis; 8151 cell growth and/or maintenance; 3700 transcription factor activity; Database ID d1srda_SCOP:b.1.8.1: Cu, Zn superoxide dismutase, SOD.	17p13.1	1/2	1/2	2/4
210816_s_at	Cytochrome b-561/CYB561HGNC; energy pathways, 6118 electron transport; integral to plasma membrane; 4128 cytochrome-b5 reductase activity;/cytochrome b561/ferric reductase transmembrane.	17q11-qter	0/1	-1/-2	-1/-2

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Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
200614_at	Heavy polypeptide (Hc) clathrin; CLTC HGNC; 6886 intracellular protein transport;5902 coated pit; 30125 clathrin vesicle coat; 5198 structural molecule activity.	17q11-qter	0/1	-1/-2	-1/-2
200029_at	Ribosomal protein L19(60S); RPL19 HGNC; 6412 protein synthesis; 5622 intracellular; 5840 ribosome; 5842 cytosolic large ribosomal subunit (sensu Eudarya); 3723 RNA binding; 3735 structural constituent of ribosome; Pathway-Ribosomal_Proteins GENMAPP/KEGG.	117q11-qter q11-qter	0/1	-1/-2	-1/-2
204995_at	Cyclin-dependent kinase 5, regulatory subunit 1(p35); CDK5R1 HGNC;cyclin-dependent protein kinase 5 activator complex; protein kinase activity,	17q12	3/8	1/2	2/4
211310_at	Enhancer of zeste homolog 1(Drosophila; EZH1 HGNC; 6350 transcription; 6355 regulator of transcription, DNA dependent; 9653 morphogenesis; 5634 nucleus; 3682 chromatin binding.	17q21.1- q21.3	6/64	6/64	5/32
205366_s_at	Homeo box B6; HOX HGNC; 6355 regulation of transcription, DNA-dependent; 7275 development; 8595 determination of anterior/posterior axis, embryo; 5634 nucleus; 3700 transcription factor activity.	17q21.3	2/4	2/4	2/4
208430_s_at	Dystrobrevin, alpha/DTNA HGNC; striated muscle contraction; signal transduction; neuromuscular synaptic transmission, cell growth and/or maintenance; 5515 protein binding; 8270 zinc ion binding.	18q12	3/8	2/2	0/1
218738_s_at	Ring finger protein 138; RNF 138 HGNC; (Full length) Hs.180403 UniGene ID Build 170 (); 5634 nucleus; 3676 nucleic acid binding; 8270 zinc ion binding	18q12.1	3/8	2/4	0/1
207315_at	CD226 antigen; CD226 HGNC; cell adhesion, signal transduction, antimicrobial humoral response (sensu Vertebrata); integral to plasma membrane; NK cell activating receptor NKP44; Myelin oligodendrocyte glycoprotein (MOG); immunoglobulin-like.	18q22.3	2/4	4/16	2/4
203022_at	Large subunit ribonuclease H2; RNASEH2A HGNC/ DNA replication, RNA catabolism, RNA binding, endonuclease activity, hydrolase activity, ribonuclease activity.	19p13.2	0/1	-1/-2	-1/-2
205382_s_at	D component of complement (adipsin)/DF HGNC 6508 proteolysis and peptidolysis; 6957 complement activation, alternative pathway; 3817 complement factor D activity; 4263 chymotrypsin activity; 4295 trypsin activity; 8233 peptidase activity; 16787 hydrolase activity; Protein Similarities: blastx P00746 Complement factor D precursor (EC 3.4.21.46) (EC 3.4.21.46)(C3 convertase activator)	19p13.3	1/2	0/1	3/8

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Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
208037_s_at	MADCAM1 HGNC; mucosal vascular addressin cell adhesion molecule 1; 6955 immune response; 7155 cell adhesion; 7165 signal transduction; 5624 membrane fraction; 16021 integral to membrane; 5515 protein binding.	19p13.3	1/2	-1	3/8
213490_s_at	MAP2K2 HGNC; mitogen-activated protein kinase kinase 2; 6468 protein amino acid phosphorylation; 5576 extracellular; 4674 protein ser/thr kinase activity; 4713 protein-tyrosine kinase activity; 5524 ATP binding; 16740 transferase activity; 4672 protein kinase activity; 5.3e-76. Pathway Integrin-mediated cell adhesion GENMAPP/KEGG; MAPK_Cascade GENMAPP/ KEGG.	19p13.3	1/2	0/1	3/8
203809_s_at	v-akt murine thymoma viral oncogene homolog 2; AKT2 HGNC; 6468 protein amino acid phosphorylation; 4674 protein serine/threonine kinase activity; 5524 ATP binding; 16740 transferase activity; Pathway Integrin-mediated_cell_adhesion; A1P_Signaling GENMAPP/KEGG.	19q13.1-q13.2	-2/-4	-1/-2	0/1
202014_s_at	Protein phosphatase 1, regulatory (inhibitor) subunit 15A; PPP1R15A HGNC; 6915 apoptosis; 6974 response to DNA damage stimulus; 7050 cell cycle arrest.	19q13.2	-2/-4	-1/-2	-1/-2
200623_s_at	Calmodulin 3 (phosphatase kinase alpha, delta; CALM3HGNC; 1539 ciliary/flagellar motility; 9288 flagellum (sensu Bacteria); 5509 calcium ion binding; 16301 kinase activity; Pathways: G13_Signaling_Pathway; G_Protein_Signaling; Glycogen_Metabolism; G13_13 Signaling_Pathway GENMAPP/KEGG.	19q13.2-q13.3	-2/-4	-1/-2	0/1
43544_at	Thyroid hormone receptor associated protein 5; THRAP5 HGNC; 6357 regulation of transcription from Pol 11 promoter; 6366 transcription from Pol 11 promoter; 6367 transcription initiation from Pol 11 promoter; 30521 androgen receptor signaling pathway; 119 mediator complex; 5634 nucleus; 3712 transcription cofactor activity; 3713 transcription coactivator activity; 4872 receptor activity; 16455 RNA polymerase 11 transcription mediator activity; 16563 transcriptional activator activity; 30374 ligand-dependent nuclear receptor transcription coactivator activity; 42809 vitamin D receptor binding; 46966 thyroid hormone receptor binding.	19q13.3	1/2	0/1	3/8
201640_x_at	Cleft lip and palate associated transmembrane protein 1: CLPTM 1 HGNC; 7275 development; 5887 integral to membrane.	19q13.3	1/2	0/1	3/8
202942_at	Beta polypeptide electron-transfer-flavoprotein; ETFB HGNC; 6118 electron transport; 5759 mitochondrial matrix; 9055 electron carrier activity; 5489 ETF beta; electron transport activity; 1.4e-128 extended.	19q13.3	1/2	0/1	3/8

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Table 1 contd.: Sample genes affected by mercury.

<i>Probe Set ID (Affymetrix)</i>	<i>Gene Title (Target Description/Symbol)</i>	<i>Chromosome Location</i>	<i>1µg/mL Hg SLR Change/ Fold Change</i>	<i>2µg/mL Hg SLR Change/ Fold Change</i>	<i>3µg/mL Hg SLR Change/ Fold Change</i>
200003_s_at	Ribosomal protein L28/RPL28 HGNC; 3735 3.2e-80 extended; Pathway: Ribosomal_proteins GENEMAPP/ KEGG.	19q13.4	-4	-7/-128	-3/-8
207857_s_at	Member 2 leukocyte immunoglobulin-like receptor, subfamily A (with TM domain); LILRA2 HGNC; 6955 immune response; 16021 integral to membrane; 4872 receptor activity; Database d1m4ka2 SCOP: b.1.4.; Killer cell inhibitory receptor.	19q13.4	-4/-16	-7/-128	-3/-8
200024_at	Ribosomal protein S5 ; RPS5 HGNC ; 6412 protein biosynthesis ; 5622 intracellular ; 5843 cytosolic small ribosomal subunit (sensu Eukarya) ; 3723 RNA binding ; 3735 structural constituent of ribosome .	19q13.4	-4/-16	-7/-128	-3/-8
217075_x_at	Transcript Description:-Consensus sequence. Grade A annotation. Representative Public ID: AF105279 NCBI; Target Description: Consensus includes gb: AF105279.1/DEF=Homo sapiens myeloid lymphoid leukemia 2(MLL2) mRNA, alternatively spliced, partial cds.	Align chro19: 40903643-40904271 (+) UCSC.	7/128	4/16	7/128
207465_at	Transcript ID-g7662575. Reported Public ID NM_014134 NCBI; gb:NM_014134.1/DEF=Homo sapiensPRO0628 protein.	Align: chr 20:39099060-39100238 (-) UCSC.	8/256	8/256	5/32
209061_s_at	Nuclear receptor coactivator 3; NCOA3 HGNC— regulation of transcription; DNA-dependent signal transduction; 6350 transcription; 6355 regulation of transcription, DNA-dependent; 7165 signal transduction; 5634 nucleus; 3713 transcription coactivator activity; 4402 histone acetyltransferase activity; 46966 thyroid hormone receptor binding. Similar to nuclear receptor coactivator 3 isoform a; amplified in breast cancer-1.	20q12	1/2	3/8	4/16
205153_s_at	Tumor necrosis factor receptor superfamily, member 5; TNFRSF5 HGNC; 6461 protein complex assembly; 6915 apoptosis; 6954 inflammatory response; 6955 immune response; 7165 signal transduction; 7275 development; 19735 antimicrobial humoral response (sensu Vertebrata); 30168 platelet activation; 42100 B-cell activation; 43123 positive regulation of I-kappaB kinase/NF-kappaB cascade; 5887 integral to plasma membrane; 4888 transmembrane receptor activity; 4872 TNFR_c6; receptor activity; 2.8e-10. Pathway: Apoptosis; Inflammatory_Response_Pathway GENMAPP/ KEGG.	20q12-q13.2	1/2	3/8	4/16
219568_x_at	SRY (sex determining region Y)-box 18; SOX18 HGNC; 6357 regulation of transcription from RNA polymerase II polymerase II promoter; 3677 DNA-binding; 3702 RNA polymerase II transcription factor activity; 5634 nucleus; 6350 transcription.	20q13.33	2/4	1/2	1/2

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Table 1 contd.: Sample genes affected by mercury.

Probe Set ID (Affymetrix)	Gene Title (Target Description/Symbol)	Chromosome Location	1µg/mL Hg SLR Change/ Fold Change	2µg/mL Hg SLR Change/ Fold Change	3µg/mL Hg SLR Change/ Fold Change
222310_at	Splicing factor, arginine/serine-rich 15; pre-mRNA splicing protein rA4 [Homo sapiens]; Regulation of nuclear pre-mRNA protein; SFRS HGNC; 3723 RNA binding; 5634 nucleus.	21q22.1	1/2	5/32	3/8
211113_s_at	ATP-binding cassette, sub-family G (WHITE), member 1/ABCG1 HGNC; cholesterol homeostasis, lipid transport, detection of hormone stimulus; response to organic substance; integral to plasma membrane; nucleotide binding; L-tryptophan transporter activity; purine nucleotide transporter activity; protein dimerization activity.	21q22.3	5/32	1/2	1/2
219433_at	BCL6 co-repressor/BCOR HGNC; Bcl-6 interacting corepressor isoform 1, 2. nasopharyngeal carcinoma susceptibility protein LZ16 mRNA.	Xp11.4	6/64	2/4	2/4
219433_at u133A	BCL6 co-repressor; immunoglobulin-binding protein G, different constituent domains; Ankyrin; myotrophin	Xp11.4	6/64	2/4	1/2
207580_at	Melanoma antigen, family B, 4/MAGEB4 HGNC.	xp21.3	5/32	1/2	4/16
206148_at	Alpha (low affinity) interleukins 3 receptor; IL3RA HGNC; 6468 protein amino acid phosphorylation; 7275 development; 16021 integral to membrane; 4872 hematopoietin/interferon-class (D200-domain) cytokine receptor activity; 5057 receptor signaling protein activity.	xp22.3 or yp11.3	-2/-4	-1/-2	-2/-4
203903_s_at	Hephaestin HEPH HGNC/ ferroxidase activity; Ceruloplasmin, multicopper oxidase, type 1; 5507 copper-binding site; 43221.1.16.3.1;ferroxidase activity; 1e-300 4322 1.16.3.1;ferroxidase activity; 9.87e-249.	Xq11-q12	3/8	1/2	0/1
202711_at	Ephrin-B1; EFNB 1 HGNC; 7155 cell adhesion; 7267 cell-cell signaling; 7275 development; 7399 neurogenesis; 5625 soluble fraction; 5887 integral to plasma membrane; 16020 Ephrin; membrane;9.e-88; 46875 ephrin receptor binding.	xq12	3/8	1/2	0/1
201994_s_at	Mortality factor 4 like 2/MORF4L2 HGNC; 1558 regulation of growth; 5634 nucleus. Similar to blastx Q9Y690 EMBL-EBI Transcription factor-like protein MORF4 (Mortality factor 4) (Cellular senescence-related protein 1) (SEN 1).	Xq22	-2/-4	-1/-2	-1/-2
200896_s_at	Hepatoma-derived growth factor (high-mobility group protein 1 -like/HDGF HGNC; 7165 signal transduction; 8283 cell proliferation; 5615 extracellular space; 5737 cytoplasm; 8083 growth factor activity 8201 heparin binding.	Xq25	-1/-2	-1/-2	-1/-2
215555_at	CDNA FLJ13712fis, clone PLACE E2000394 Hs.549748 NCBI	TBC*	8/256	7/128	8/256
220222_at	PRO1905 HGNC; Hypothetical protein.	TBC*	7/128	4/16	5/32

SLR-- signal log ratios

*TBC = To be confirmed

Our results therefore show that mercury dose-dependently stimulates or down regulates most genes located on all human chromosomes including genes involved in apoptosis and metabolic regulations with significant effects on genes involved in the immune system pathways. Some of the leading genes affected by mercury exposure are highlighted in red/blue in the Ingenuity systems analysis (IPA) pathways kindly provided by IPA Inc (Figures 1-4). The IPA network programs identify genes affected by mercury exposure and reveal their possible role and relationship to other genes operating in common pathways. These procedures can identify possible pathologies resulting from metabolic dysfunctions and disease development.

Figures 1-3 are computer generated IPA genetic pathways showing the cell cycle, B cell receptor and p38 MAPK signaling routes that are influenced by genes that are affected by mercury exposure. These diagrams demonstrate mercury's possible influence on several metabolic pathways. Red coloration indicates gene upregulation while blue coloration indicates inhibition of gene expression on exposure to mercury. Mercury

exposure leads to effects on several of biochemical pathways involving products of genes in cell cycle signaling: G2/M checkpoint regulation, TGF-β, IGF-1, insulin receptor activity, chemokine, Wnt/ β-catenin, integrin, PPAR, SAPK/JNK, JAK/Stat, B and T cell receptor, G-protein-coupled receptor, IL-2, ERK/MAPK, death receptor signaling such as apoptosis, NF-κB, cell cycle and above all immune responses regulated by most of these genes. Gene influences on each other as revealed by Ingenuity Pathway Analysis (IPA) demonstrate that mercury is likely to exert a high effect on transcription factors. Further analyses of these genetic pathways and the implications of the immune system regulation will be addressed in subsequent communication. IPA diagrams also point to the role of several genes linked to associated metabolic networks that are subject to mercury toxicity (Figure 5). This exemplifies the potential for mercury to affect susceptible individuals with the right MHC haplotype combinations and who are prone to develop autoimmune and/or other diseases commonly linked with mercury exposure from the environment.

Cell Cycle: G1/S Checkpoint Regulation

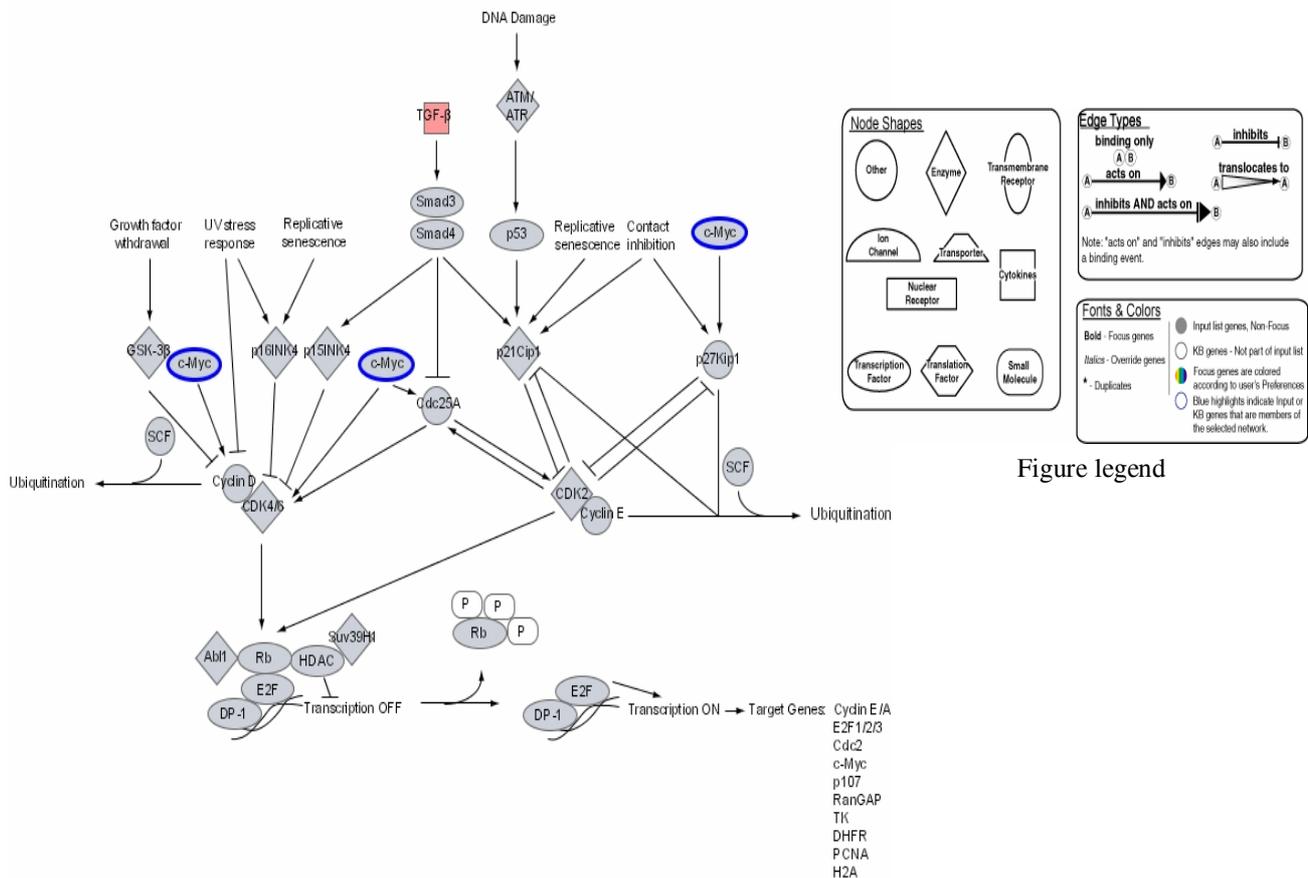


Figure1: Modulation of cell cycle G1/S checkpoint regulation by mercury: Activation of TGF-β and repression of C-Myc.

B Cell Receptor Signaling

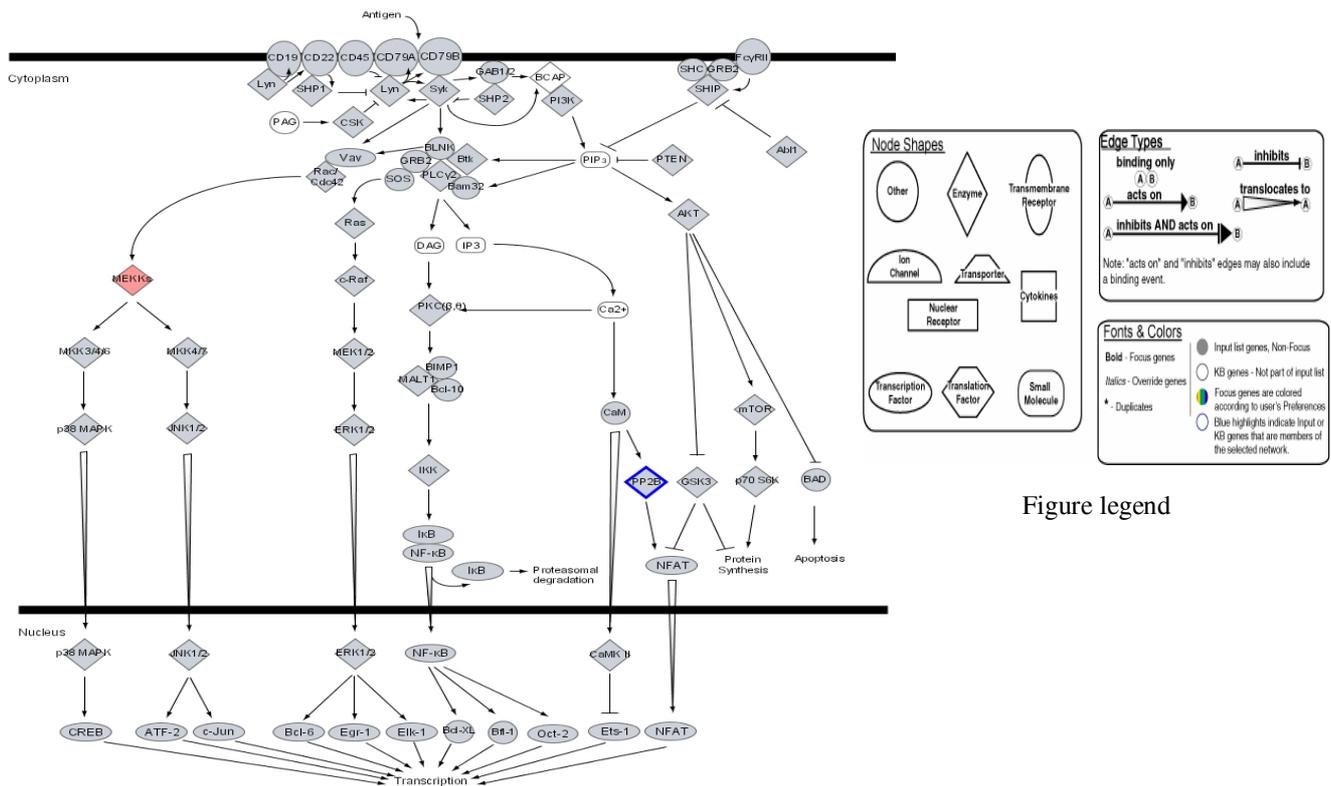


Figure 2: Modulation of B cell cycle receptor signalling by mercury: Activation of MEKK and repression of PF 2B.

p38 MAPK Signaling

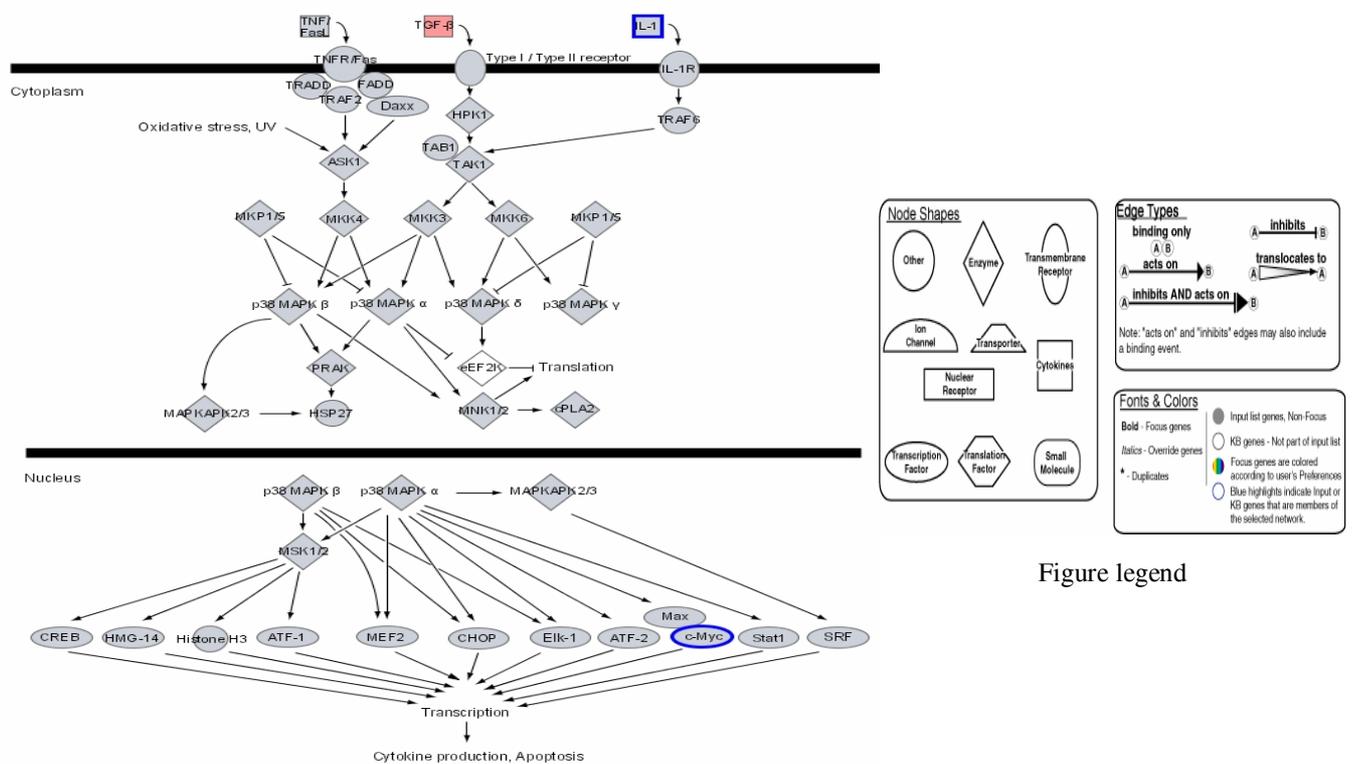


Figure 3: Modulation of P38 MAPK signalling by mercury: Activation of TGF-β and repression of IL-1 and c-Myc.

T-Cell Antigen Receptor Signaling

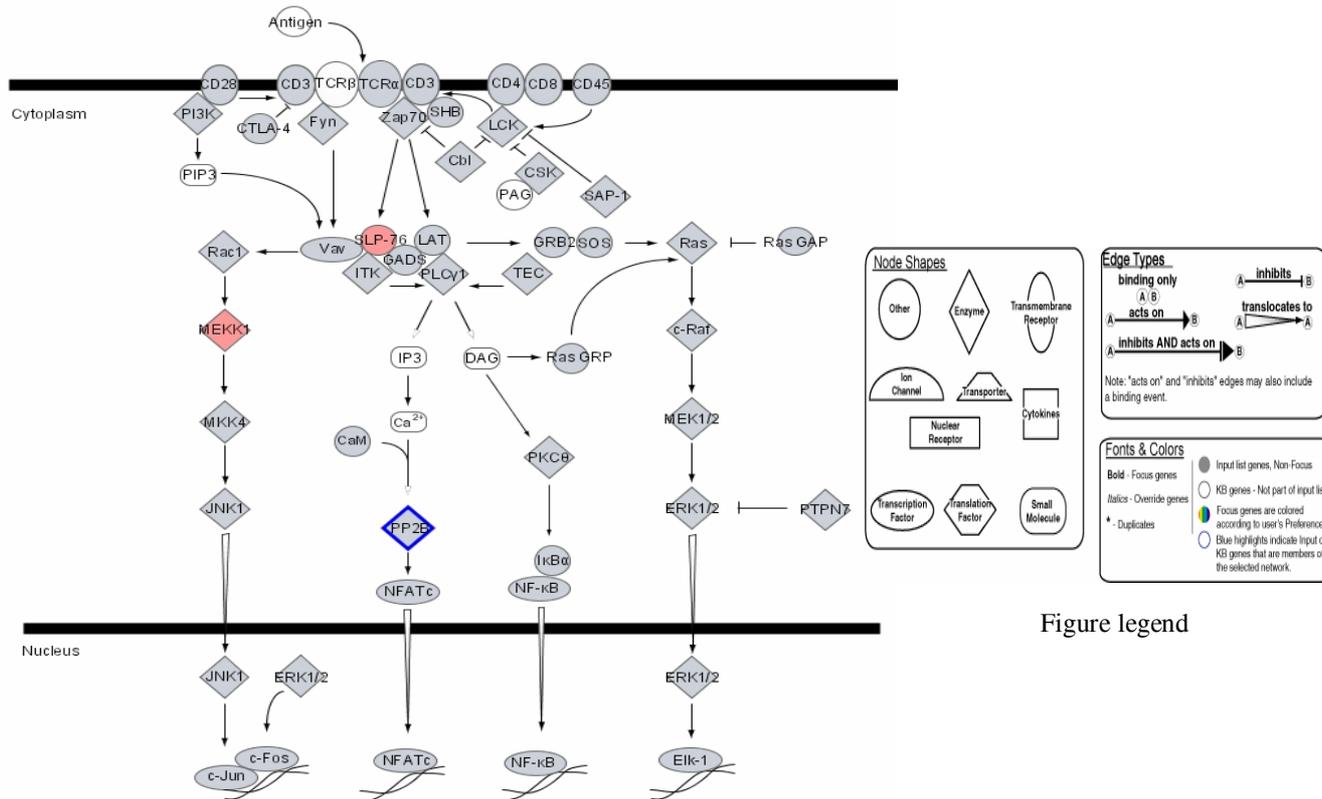


Figure legend

Figure 4: Modulation of T-Cell antigen receptor signalling by mercury: Activation of SLP-76 and MEKK, and repression of PP-2E.

Discussion

This study shows that several genes in haplotype combinations are subjected to pronounced changes on exposure to environmental mercury. Among these genes are transforming growth factor beta (TGF-β) superfamily of cytokines (Figure 1). This group of family genes is associated with regulating the cell cycle essentially for maintenance of normal immunological homeostasis and lymphocyte proliferation. Proteins synthesized from these genes play important roles in regulating essential cellular functions such as differentiation and apoptosis. Our findings indicate that transforming factor beta (TGF-β) superfamily of cytokines is over expressed on mercury exposure. Some cells, lymphocytes among them are known to respond to TGF-β by undergoing apoptosis. Apoptosis may lead up to accumulation of self antigens within a localized part of the body and break the body's immunological tolerance giving rise to autoimmune induction. The mechanisms regulating this process are yet to be clarified. The multifunctional effects of TGF-β superfamily of cytokines involve oligomeric complex formation of type I and type II serine/threonine kinase receptors. TGF-β binding to primarily the type II (T^βRII) stabilizes the heteromeric complex with the type I receptor (T^βRI) that results in transphosphorylation and activation of T^βRI by the T^βRII. Activated T^βRI transmits the signal through

transient interaction with and phosphorylation of receptor-restricted Smads. Smad2 and Smad3 mediate TGF-β and activin signals, while Smad1, Smad5, and Smad8 act downstream of bone morphogenetic proteins [65-67]. Receptor-mediated phosphorylation takes place in two serine residues in a SXSXS motif in the C-terminal domain of receptor-restricted Smads. On phosphorylation, receptor-restricted Smads dimerize with Smad4 and translocate to the nucleus where it modulates transcription of TGF-β target genes. TGF-β is known to be a potent inducer of apoptosis in B-lymphocytes and is essential for immune regulation and maintenance of self-tolerance. Induction of Smad3 and/or Smad4 transcription factors by the interaction of TGF-β and receptors also leads to release of tissue factors (TFs) involved in the transcription of Cdc25A cyclin proteins as well as p15INK4 that in turn transcribe CDK4 and CDK6 cyclins responsible for the stimulation of Rb and E2F transcription factors. Combination of E2F TF DP-1 after release of phosphorylated Rb turns on TFs that have a wide array of effects on target genes in the cell cycle. Included in this target genes are cyclin E/A, E2F1/2/3, Cdc2, c-myc, p107, RanCAP, TK, DHFR, PCNA and H2A products (Figure 5). Transcription factor products of Smad3 and Smad4 that stimulate Cdc25A leads to synthesis of CDK2 enzymes coupled to cyclin E groups in the pathway of p53 and c-myc necessary for DNA repair processes through ubiquitination [65].

The growth inhibitory and apoptotic effects of TGF- β and anti-IgM have been found to both block cells in late G₁ with decline in the level of c-myc, and both modulate the activity of Rb, p53, p21Waf1/Cip1, p27, cyclin A, caspase protease family members, and NF κ B and thereby induce cells to growth arrest and apoptosis [60, 61]. Over expression of TGF- β cytokines induced by mercury may lead to transcription of Smad6 and Smad7; these molecules act as inhibitors of TGF- β family signaling. Smad7 interacts through binding with T β RI to block the phosphorylation and activation of receptor-restricted Smads that stops further signal propagation [60, 61]. Above all Smad7 expression has been shown to be induced by other factors such as interferon- γ , and TNF- α , epidermal growth factor, phorbol ester, and shear stress, indicating that transcription of Smad7 can serve as regulatory means of integrating and modulating the physiological response of different signaling pathways [62-64]. During developmental period the clonal deletion of self-reactive B cells mediated through apoptosis is necessary for maintenance of tolerance. Failure to eliminate immature B cells has the consequence of autoimmune diseases and cancer development [65]. Several aberrant functions associated with many pathways involving the cell cycle and the immune responses are therefore possible through intoxication with mercury.

Haplotype structure is often a better predictor of phenotypic consequences than are individual polymorphisms [56] and help relate genetic background to disease progress, diagnosis and/or treatment. A haplotype is made up of a group of nearby alleles that are inherited together. Because of their close proximity these genes tend to be better associated with patterns of diseases and by inference responses to drugs as well. To use haplotype as diagnostic and/or disease management tool it would be desirable to develop simple but vigorous molecular methods to determine the haplotype structure of patients [66]. Several evidences give support to complex makeup of genetics of autoimmune diseases such as lupus; haplotype links may be ideal diagnostic tool for disease managements.

Systemic lupus erythematosus (SLE) is an example of chronic autoimmune disease with female predominance (>90%), particularly in the child-bearing years. African-Americans are three times affected than European-Americans. Clinical features of SLE show extreme heterogeneity, varying in pathogenicity from mild forms of the disease to an advanced relentlessly progressive end organ damage. Epidemiological findings point to strong genetic component for susceptibility to SLE [64, 65] and multiple genes including those affecting immune complex deposition and the MHC [70, 71]. Many whole-genome linkage studies so far suggest about 20 SLE susceptibility loci [65, 67] indicating that SLE is polygenic with contributions from multiple genes, each one of which has a degree of effect. Genetic makeup seems to differ among racial groups. Out of the 20 potential SLE loci identified only three are thought to be of combined pedigrees rather than European-American or African-American pedigrees separately. Genome scan of 94 extended multiplex pedigrees by the use of model-based linkage analysis identified potential SLE loci at chromosomes 1q41, 1q23, and 11q14-23 in

African-Americans; 14q11, 4p15, 11q25, 2q32, 6q26-27, 12p12-11 and 19q13 in European-Americans; and 1q23, 1q31, 13q32, and 20q13 in all pedigrees combined [69].

There was strong evidence that linkage in the African-American pedigrees was located in chromosomes 1, 3, and 11. An effect for the Fc γ RIIA candidate polymorphism at 1q23 in African-Americans is syntenic with linkage in a murine model of lupus. Sib pair and multipoint nonparametric analyses also support linkage ($p < 0.05$) at nine loci detected by two-point lod score analysis (lod >2.0). These results confirm the complexity associated with genetic susceptibility to SLE and point to the racial origin of the specific influence of these genetic effects. Our findings reinforce these data. Mercury seems to influence specific regulation of most of these genes aforementioned. For Afro-American group several of the genes influenced by mercury exposure involve genes expressed on *chromosome 1* (1p13.1, 1p21, 1p21.3, 1p31, 1p32-31, 1p33, 1p34.2, 1p34.3, 1p34, 1p36.22, 1p36.33, 1p36.33-p36.12, 1q12-q21, 1q21, 1q21.2-q21.3, 1q21-q23, 1q23, 1q23.1, 1q23.3, 1q24.2, 1q24, and 1q25.1-q31.1), *chromosome 3* (3p21.3, 3p21.2-p21.1, 3p21.31, 3p21-p12, 3p21, 3q13.31, 3q23 and 3q27.3), and *chromosome 11* (11p15.1-p14, 11p15.4, 11q22 and 11q23.3). For European-Americans our result show that the following genes may be prominent in mercury toxicity: *chromosome 2* (2p11, 2p11.2, 2p21, 2p22.1, 2p24.2, 2p25, 2q33, 2q34, 2q33-q35, 2q36-q37, 2q37.3), *chromosome 4* (4p12, 4p16, 4p16.1, 4q24, 4q33-q34, 4q34.1), *chromosome 6* (6p21.3, 6p22.1, 6p24.3, and 6q16), *chromosome 11* (11p15.1-p14, 11p15.1, 11p15.4, and 11q23.3), *chromosome 12* (12p11.23, 12q15-q21, 12q21.1, 12q21.2, 12q22, and 12q24.2), *chromosome 14* (14q, 14q23.3, and 14q32.33), and *chromosome 19* (19p13.2, 19q13.2, 19p13.3, 19q13.2-19q13.3 and 19q13.4).

Such wide effects of mercury translate to risk associations when disease susceptibility is considered. This means that it is only at the right genetic combinations and the appropriate line-up of associated genes that disease susceptibility ensues. That goes to argue for severity of disease as well. Mercury-exposed individuals carrying the appropriate allelic-combinations located on specific haplotypes are prone to develop autoimmune diseases. Our findings give room to emphasize that high SLE prevalence in Blacks as compared to Caucasians, may be explained on haplotype basis. This is because quantitatively exons of chromosomes 1 and 3, known to be highly involved in immune response regulations and highly found among blacks may be highly affected by mercury. Hence the use of microarray techniques combined with MHC haplotype analysis can help to elucidate pathways of autoimmune disease pathogenesis and risk assessment, as well as to identify targets for drug therapy.

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