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 sulphydryl 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 (1-3 µg/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 immuno-stimulant 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 Hg₂⁺ 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-κB 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-γ 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 transription. Two-signal transduction pathways that may be considered important in the rheumatoid synovium are the AP-1 and the NF-κB pathways [11, 51]. NF-κB seems to be particularly important in chronic inflammatory diseases, both in mediating IL-1 and TNFα production as well as in mediating their effects on target cells after binding to cell surface receptors. Stimulation of AP-1s and the NF-κB 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 different types and levels of environmentally available 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: Fast (CD95/L), Sap (serum amyloid P-component), FcγR2b (FcγRIIB), Cr2 (CD21/CD35) and Pippc (CD45), whereas deficiency of FcεRIγ (Fcγ-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 (1-3µg/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. RNase, RNA later RNA stabilization kits for RNA extraction were purchased from Qiagen (Qiagen) was then added for RNA purification procedures to circumvent electrophoretic runs for monitoring extracted RNA purity.

Total RNA Extraction, Stabilization and Quantitation

Cells stored in RNA later 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. Enriched 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 µL) 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 µg/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 µm pixel values, at wavelength 570 nm for 50 µm probe arrays with probe cells 24 µm 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 µg/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 (γ1) and gamma 2 (γ2) 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 hepatopoietin/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

| 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 |
|---------------------------|----------------------------------------|---------------------|----------------------------------|----------------------------------|----------------------------------|
| 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 EMBLEBI-Transforming growth factor beta (TGFb), N-terminal Pfam-IPR001839 EMBLEBI -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 hematopoiein/ 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.

| Probe Set ID (Affymetrix) | Gene Title (Target Description/Symbol)                                                                 | Chromosome Location | SLR Change/Fold Change | SLR Change/Fold Change | SLR Change/Fold Change |
|---------------------------|-------------------------------------------------------------------------------------------------------|---------------------|------------------------|------------------------|------------------------|
| 208628_s_at               | Nuclease sensitive element binding protein 1; NSEPI 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 chromosome 1: 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.

| 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 |
|---------------------------|----------------------------------------|---------------------|---------------------------------|---------------------------------|---------------------------------|
| 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 (translocon-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-B) Fc-γ RIIIB), (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.

| 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 |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|---------------------------------|---------------------------------|---------------------------------|
| 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 binging                                                                                                                                  | 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: phosphotidylinositol signaling system GENMAPP/KEGG.                                 | 2q33                | 0/1                             | -2/-4                           | 2/4                             |

SLR-- signal log ratios
Table 1 contd.: Sample genes affected by mercury.

| Probe Set ID (Affymetrix) | Gene Title (Target Description/Symbol) | SLR Change/Fold Change | SLR Change/Fold Change | SLR Change/Fold Change |
|---------------------------|---------------------------------------|------------------------|------------------------|------------------------|
| 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 1V, 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 | Change/Fold | Change/Fold | Change/Fold |
|---------------------------|-----------------------------------------------------------------------------------------------------|---------------------|-------------|-------------|-------------|
| 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 PCRs_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       |
| 201009_s_at               | ADP-ribosylation factor 4/ARF4 HGNC                                                                 | 3p21.2-p21.1       | -3/-8       | -2/-4       | -1/-2       |
| 39966_at                  | Chondroitin sulfate proteoglycan 5 (neurogliocan 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; XLHRSF-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.

| 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 |
|---------------------------|----------------------------------------|---------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| 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 |

SLR-- signal log ratios
Table 1 contd.: Sample genes affected by mercury.

| Probe Set ID | Gene Title | Chromosome Location | SLR | Change/Fold Change | SLR | Change/Fold Change | SLR | Change/Fold Change |
|--------------|------------|---------------------|-----|---------------------|-----|---------------------|-----|---------------------|
| 216720_at    | Cytochrome P<sub>450</sub>, family 2, 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 AF118086 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 B1 (HLA-DQB1 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 |

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 SLR Change/Fold Change | 2µg/mL Hg SLR Change/Fold Change | 3µg/mL Hg SLR Change/Fold Change |
|---------------------------|--------------------------------------|---------------------|---------------------------------|---------------------------------|---------------------------------|
| 214509_at                 | Histone 1, H31. Transcripts alignment(s)-NM_003533NCBI-Homo sapiens histone 1, H3i (HIST1H31), mRNA 11/11 | 6p22-p21.3          | 0/1                             | 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 HGNC | 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. *Alignment with chromosomes 1-3, 5-21, and X.* | 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.

| Probe Set ID | Gene Title (Target Description/Symbol) | Chromosome Location | 1µg/mL Hg | 2µg/mL Hg | 3µg/mL Hg |
|--------------|----------------------------------------|---------------------|-----------|-----------|-----------|
| 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; 7217 tachykinin 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. | 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 |

SLR-- signal log ratios
Table 1 contd.: Sample genes affected by mercury.

| Probe Set ID (Affymetrix) | Gene Title (Target Description/Symbol) | Chromosome Location | SLR Change/Fold Change | SLR Change/Fold Change | SLR Change/Fold Change |
|---------------------------|----------------------------------------|---------------------|------------------------|------------------------|------------------------|
| 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; 9p13.2-p13.1 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.

| Probe Set ID (Affymetrix) | Gene Title | Chromosome Location | SLR Change/ Fold Change | SLR Change/ Fold Change | SLR Change/ Fold Change |
|---------------------------|------------|---------------------|-------------------------|-------------------------|-------------------------|
| 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 phosphorylstion; 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 myristolation; 9249 protein lipolation; 4379 glycylpeptide 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; momoamine_GPCRs GENMAPP/KEGG | 10q21-q24 | 8/256 | 6/64 | 4/16 |

SLR-- signal log ratios
Table 1 contd.: Sample genes affected by mercury.

| Probe Set ID (Affymetrix) | Gene Title (Target Description/Symbol) | Chromosome Location | SLR Change/Fold Change | SLR Change/Fold Change | SLR Change/Fold Change |
|--------------------------|---------------------------------------|---------------------|------------------------|------------------------|------------------------|
| 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 benimidazoles 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.

| 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 |
|--------------------------|-------------------------------------------------------------------------------------------------------|---------------------|----------------------------------|----------------------------------|----------------------------------|
| 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 ubiquination; 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 microirbll; 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 phosphotidylinositol 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.

| 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 |
|---------------------------|---------------------------------------|---------------------|----------------------------------|----------------------------------|----------------------------------|
| 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.

| 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 |
|---------------------------|----------------------------------------|---------------------|---------------------------------|---------------------------------|---------------------------------|
| 216892_at                | Immunoglobulin heavy locus/IGH@ HGNC; IGHV3 protein (Fragment); CDNA FLJ25997 fis, clone DMC06923, highly similar to Ig gamma-1 chain 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- embl 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 musc). | 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                           |

SLR-- signal log ratios
Table 1 contd.: Sample genes affected by mercury.

| Probe Set ID (Affymetrix) | Gene Title (Target Description/Symbol)                                                                                                                                                                                                 | Chromosome Location | SLR Change/Fold Change | SLR Change/Fold Change | SLR Change/Fold Change |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|------------------------|------------------------|------------------------|
| 200614_at                | Heavy polypeptide (Hc) clathrin; CLTC HGNC; 6886 intracellular protein transport; 5902 coated pit; 30125 clathrin vesicle coat; 5198 structural molecule activity.                                                                                                                              | 17q11-pter          | 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-pter q11-pter | 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              | Homebox 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 prolylase 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                    |

SLR-- signal log ratios
| Probe Set ID (Affymetrix) | Gene Title (Target Description/Symbol) | Chromosome Location | 1µg/mL Hg Change/Fold Change | 2µg/mL Hg Change/Fold Change | 3µg/mL Hg Change/Fold Change |
|---------------------------|---------------------------------------|---------------------|-----------------------------|-----------------------------|-----------------------------|
| 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 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. | 19q13.1-13.2       | -2/-4                       | -1/-2                       | 0/1                         |
| 202014_s_at               | Protein phosphatase 1, regulatory (inhibitor) subunit 15A; PPI1R15A 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. | 19q13.2-13.3       | -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                         |

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 |
| 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; 4696 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 TNFRR_c6; receptor activity; 2.8e-10. Pathway: Apoptosis; Inflammatory_Response_Pathway GENEMAPP/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 |

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 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 FLJ13712fs, 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-kB, 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: GI/S Checkpoint Regulation**

**Figure 1:** Modulation of cell cycle GI/S checkpoint regulation by mercury: Activation of TGF-β and repression of C-Myc.
**Figure 2:** Modulation of B cell cycle receptor signalling by mercury: Activation of MEKK and repression of PF 2B.

**Figure 3:** Modulation of P38 MAPK signalling by mercury: Activation of TGF-β and repression of IL-1 and c-Myc.
**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-\(\beta\)) 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-\(\beta\)) superfamily of cytokines is over expressed on mercury exposure. Some cells, lymphocytes among them are known to respond to TGF-\(\beta\) 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-\(\beta\) superfamily of cytokines involve oligomeric complex formation of type I and type II serine/threonine kinase receptors. TGF-\(\beta\) binding to primarily the type II (T\(^\beta\)RII) stabilizes the heteromeric complex with the type I receptor (T\(^\beta\)RI) that results in transphosphorylation and activation of T\(^\beta\)RI by the T\(^\beta\)RII. Activated T\(^\beta\)RI transmits the signal through transient interaction with and phosphorylation of receptor-restricted Smads. Smad2 and Smad3 mediate TGF-\(\beta\) 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 SSXS 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-\(\beta\) target genes. TGF-\(\beta\) 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-\(\beta\) 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].

Figure 4: Modulation of T-Cell antigen receptor signalling by mercury: Activation of SLP-76 and MEKK, and repression of PP-2E.
Figure 5: Genes and molecular pathways affected by mercury in HepG₂ cells (A: Network 1; B: Network 2)
The growth inhibitory and apoptotic effects of TGF-β and anti-lgM 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 NFXB 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 TβRI 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 a 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 rigorous 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 management.

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; 1q41, 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 FcγRIIA 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, 1q32.2, 1q32.3, 1q32.33, 1q21-2, 1q21.2-21.3, 1q21-22, 1q23, 1q23.1, 1q23.3, 1q24.2, 1q24, and 1q25.1-25.1). chromosome 3 (3p21.3, 3p21.2-p21.1, 3p21, 3p21-p12, 3p21, 3q13.31, 3q23 and 3q23.7), and chromosome 12 (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 (1p11, 1p11.2, 2p21, 1p22.1, 2p22.2, 2p22.3, 2p23, 2p23.3, 2q32, 2q32.3, 2q32.33, 2q36-37, 2q37.3, 3q26, 3p13.1, 4p12, 4p16, 4p16.1, 4q24, 4q34-q35, 4q34), chromosome 4 (4p12, 4p16, 4p16.1, 4q24, 4q34-q35, 4q34.1), chromosome 6 (6p21.3, 6p21.1, 6p21.3, 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, 19q13.2-3.3 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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