



In vitro screening for chemical toxicity in a genetically-diverse human model system

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Abstract

Immortalized human lymphoblastoid cell lines have been used to demonstrate that genetic polymorphisms control gene expression, that it is possible to use cell lines from related and unrelated individuals to identify the factors that affect the phenotypes in response to xenobiotic treatment, and that there is heritability of gene-expression traits in segregating human populations. Our research aims to extend the application of such studies to investigative toxicology by assessing inter-individual variability and heritability of chemical-induced toxicity phenotypes in cell lines from the Centre d'Etude du Polymorphisme Humain (CEPH) trios assembled by the HapMap Consortium. Our goal is to aid in the development of predictive *in vitro* genetics-anchored models of chemical-induced toxicity. In our initial screen we treated 87 cell lines from the CEPH trios with 14 environmental chemicals. We applied each chemical in three doses with a replicate of each dose on a 96-well plate, including wells for background and controls. We assessed production of ATP, a measure of cell viability, and caspase-3/7 activity, a marker of apoptosis, 24 hours after treatment. We also produced biological replicates for a sub-sample of the cell lines to evaluate reproducibility of both assays. This experiment demonstrated that variability of response across the chemicals exists for some, but not all agents, with perfluorooctanoic acid and phenobarbital exhibiting the greatest degree of inter-individual variability. At the same time, an appreciable degree of inter-individual variability in susceptibility of cell lines to the chemicals was also observed. While our preliminary assessment of the data shows no significant heritability of toxicity response phenotypes across these cell lines, genetic factors controlling wide variability in response to some agents needs to be addressed. In summary, we show that the approach of screening chemicals for toxicity in a genetically-defined, yet variable *in vitro* system is potentially useful for identification of both agents and individuals that may be at highest risk.

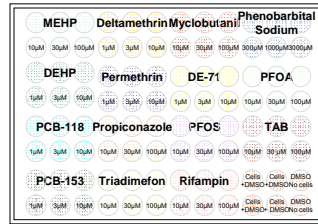


Figure 1. Plate Design. Abbreviation Key: MEHP, Mono-2-ethylhexylphthalate; DEHP, Di(2-ethylhexyl)phthalate; PCB, Polychlorobiphenyl; DE-71, Penta-bromodiphenyl oxide; PCN, Pregnenolone-16 α -carbonitrile; PFOA, Perfluorooctanoic acid ammonium salt; PFOS, Perfluorooctanesulfonic acid potassium salt; TAB, Tetraoctyl ammonium bromide.

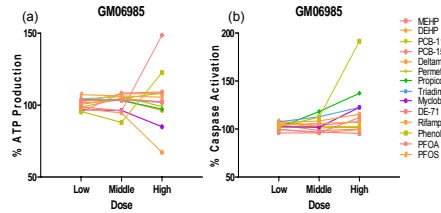


Figure 2. Sample Dose-response Curves. These curves represent the response (% of control) of one of the cell lines (GM06985) to all 14 chemicals at three different doses (see Figure 1 for dose information). Each line represents one chemical as shown in the legend. In (a) the ATP assay there is both an increase and a decrease in ATP production, while in (b) the Caspase assay there is only an increase in caspase 3/7 activation.

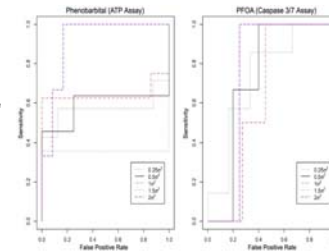


Figure 4. Determining significance thresholds in responses using ROC analysis. ROC curves were created by applying different weights to the experimental variation coefficient and classifying individuals based on one of the experimental runs with a second experimental run used to validate the classification. Curves with the largest area beneath them indicate superior classification as compared to the thresholds tested. For this study, we chose a threshold of 2 standard deviations to classify individual cell lines as 'responders'.

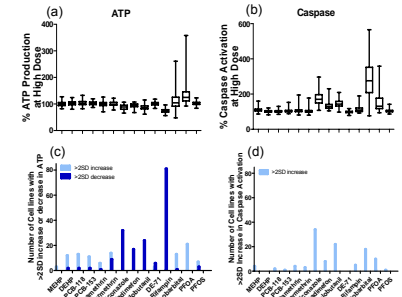


Figure 5. Extent of inter-individual variability of response to 14 chemicals. Box-and-whiskers plots (a) and (b) demonstrate variability in responses to chemicals at highest dose. Bar graphs (c) and (d) show the number of cell lines that exhibited a response greater than 2 standard deviations above or below 100% of corresponding control.

Introduction

- Variation in inherited genetic susceptibility is known to play an important role in responses to toxic agents;
- Datasets which combine high-density genotyping, together with traditional toxicity data, and gene expression profiling enable the discovery of genetic causes of susceptibility;
- Recent studies have highlighted the use of HapMap lymphoblasts for generation of GxE data *in vitro* (Dolan et al., 2004; Watters et al., 2004; Jen and Cheung, 2003);
- CEPH trios (i) provide an accurate representation of genomic DNA from a diverse population; (ii) represent a large, renewable resource for a wide range of applications (Meucci et al., 2005); and (iii) have been densely genotyped (>5x10⁶ SNPs) which allows for association mapping of the phenotypic differences between subjects;
- Combining genetic inheritance information for both molecular profiles and complex traits is a promising strategy for understanding which genes, pathways, and biological processes are also under the influence of a given QTL;
- While HapMap cell lines have important limitations, we performed experiments on this population-wide human resource with the goal of assessing inter-individual variability and/or heritability of chemical-induced toxicity phenotypes.
- We aim to use these phenotypes in future studies to (i) identify and validate regulatory networks that can be used to prioritize genotype-phenotype studies and/or chemical agents; and (ii) determine the heritability of gene expression and master regulators of the expression and other toxicity-related phenotypes.

Methods

- 87 CEPH cell lines (29 trios) were obtained from Coriell Cell Repository;
- Cells were cultured in RPMI media (Gibco) supplemented with 1% Pen/Strep (Gibco) and 15% FBS (Hyclone) and grown to 10⁶ cell/ml and viability of 85% or higher;
- 14 chemicals (provided by the EPA, see Figure 1) were aliquoted in three doses with a replicate of each dose on a 96-well plate, including wells for background and controls.
- 10⁴ cells/well were added to the plate of chemicals and incubated for 24 hrs at 37°;
- ATP production, a measure of cell viability, was assessed using a Cell-Titer-Glo Luminescent Cell Viability Assay (Promega), and caspase-3/7 activity, a marker of apoptosis, was measured using Caspase-Glo 3/7 Assay (Promega);
- 85 of 87 of the cell lines and a subset of 15 biological replicates were completed for the ATP assay. 84 of 87 cell lines and a subset of 13 biological replicates were completed for the Caspase assay.

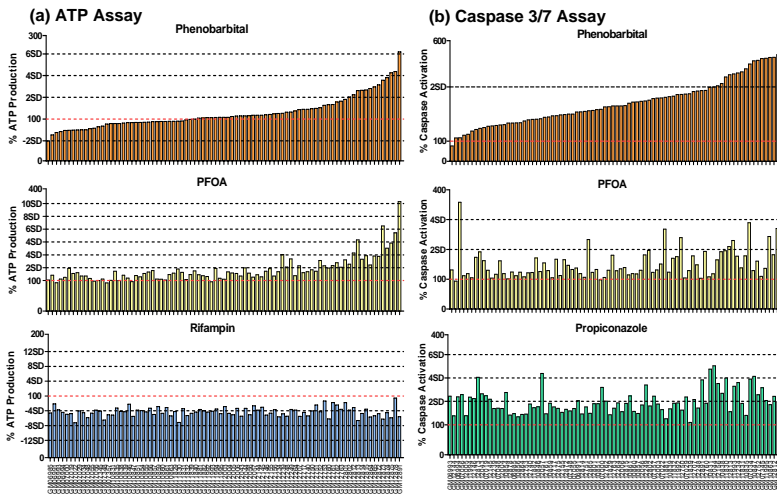


Figure 3a. Representative plots of inter-individual variability in response to chemicals (ATP assay). Cell lines were sorted (lowest to highest) using ATP levels (% of control) as a phenotype in response to treatment with the highest dose of Phenobarbital. The cell lines were then plotted in the same order for response to PFOA and Rifampin. The red line indicates 100% of control and the y-axis are scaled to show standard deviation increments (determined from replicate experiments).

Figure 3b. Representative plots of inter-individual variability in response to chemicals (Caspase 3/7 assay). Cell lines were sorted (lowest to highest) using Caspase 3/7 activity (% of control) as a phenotype in response to treatment with the highest dose of Phenobarbital. The cell lines were then plotted in the same order for response to PFOA and Propiconazole. The red line indicates 100% of control and the y-axis are scaled to show standard deviation increments (determined from replicate experiments).

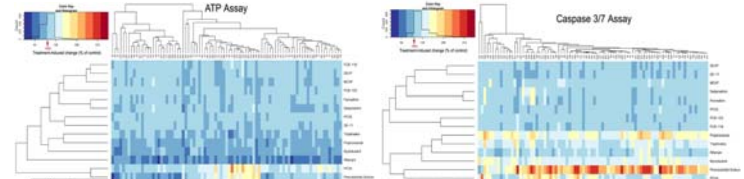


Figure 6. Correlation plots show similarities in responses between chemicals and individuals. Heatmaps were generated by clustering (average linkage on the correlation metric) the data on % ATP production and % Caspase activation at highest dose across all chemicals and individuals. The heatmaps are ordered both vertically and horizontally with the most correlated participants and chemicals being displayed near to one another. Data was mean-centered and transformed into the color palette as indicated in the color inserts.

Conclusions

- Variability of responses across the 14 chemicals tested exists for some, but not all agents, with perfluorooctanoic acid, phenobarbital and propiconazole exhibiting the greatest degree of inter-individual variability.
- Significant inter-individual variability in susceptibility of cell lines to this set of chemicals was observed.
- Preliminary assessment of the data shows no appreciable level of heritability of toxicity phenotypes across these cell lines.
- Genetic factors controlling wide variability in response to some agents needs to be further addressed.
- The approach of screening chemicals for toxicity in a genetically-defined, yet variable *in vitro* system is potentially useful for identification of both agents and individuals that may be at highest risk.

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Acknowledgements

This work was supported, in part, by the Carolina Center for Computational Toxicology through a grant from US EPA RD-83382501. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy