Genomic Approaches to Adverse Drug Reactions in Children

Dr. Bruce Carleton
BC Children’s Hospital
University of British Columbia
Child & Family Research Institute
Case Studies

**Case 1**
- 14 yrs old
- Osteosarcoma of Right proximal tibia
- Diagnosed Nov 2000
- Chemotherapy:  
  - Cisplatin
  - Doxorubicin
  - Methotrexate
- Alive and Well

**Case 2**
- 12 yrs old
- Osteosarcoma of Right Proximal tibia
- Diagnosed Oct 1998
- Chemotherapy:  
  - Cisplatin
  - Doxorubicin
  - Methotrexate
- Alive and Well
Audiogram

Case 1

Baseline Audio - ONCOLOGY

Case 2

BASELINE STUDIES
Case 1

Case 2

MIDPOINT OF THERAPY
(AFTER 2 CYCLES OF CISPLATIN)
Case 1

CURRENT STUDIES

Case 2
Case Studies

Cases sound similar
- Same tumor
- Same protocol
- Same good outcome from cure point of view

However:
- Significant difference in Audiograms
- Case 2 needed last 2 doses of cisplatin held due to significant hearing loss
- Case 2 needs hearing aids
Why does one child get hearing loss with cisplatin, while another does not?
Individual variability in drug response can have serious consequences

Stevens-Johnson Syndrome (SJS)
Adverse Drug Reaction
Adverse Drug Reactions

- 4-6th leading cause of death in the USA\(^1\)
- Health care costs: $137-177 billion annually (USA)\(^2-3\)
- Cause 7% of all hospital admissions\(^4\)
- Cause serious reactions in over 2,000,000 hospitalized patients (6.7%) each year in the USA\(^1\)
- Cause fatal reactions in over 100,000 hospitalized patients each year in the USA\(^1\)
- 50% of newly approved therapeutic health products have serious ADRs, discovered only after the product is on the market (Health Canada, 2007)
- 95% of all ADRs are unreported

5. MjoÈmdal et al, EACPT3, 1999
6. Moore et al., 2007
Pharmacogenomics

Avoid adverse drug reactions

Maximize drug efficacy for individual patients

Pharmacogenetic Profile:

High risk of ADR (50%):
treat with alternative drug or dose

Moderate risk of ADR (12.5%):
treat with alternative drug or dose

Low risk of ADR (0%):
treat with conventional dose

All Patients with Same Diagnosis

10% risk of adverse reaction
WE CAN’T TREAT CHILDREN LIKE ADULTS

Increased Risk of Severe ADRs in Children

- >75% of approved drugs used in children are untested in pediatric populations
- Young children cannot evaluate or express their own response to medications
- Pediatric dosage forms not available
- Children metabolize drugs differently than adults
Variability in Drug Metabolites in Childhood Despite Administration of Equivalent Doses

e.g. Valproic Acid

- Increased CYP2A6, CYP2C9 activity in children
- Increased formation of hepatotoxic metabolite in children
Targeted Active ADR Surveillance and Pharmacogenomics: The GATC Project

Genotypic Adjustment of Therapies in Childhood

Project Leaders: Bruce Carleton & Michael Hayden
Adverse drug reaction active surveillance: developing a national network in Canada’s children’s hospitals†‡

BC Carleton PharmD†, RL Poole PharmD‡, MA Smith MSc†, JS Leeder PharmD, PhD†, R Ghannadan BSc†, CJD Ross PhD‡, MS Phillips PhD∥
and MR Hayden MB, ChB, PhD, FRCP (C), FRSC∥

†Pharmaceutical Outcomes Programme, Children’s and Women’s Health Centre of British Columbia, Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada
‡Department of Pediatrics, Neonatal and Developmental Medicine, Lucile Packard Children’s Hospital, Stanford University Medical Center, Palo Alto, CA, USA
∥Department of Pediatrics, Division of Clinical Pharmacology and Medical Toxicology, Children’s Mercy Hospitals and Clinics, Kansas City, MO, USA
∥Centre for Molecular Medicine and Therapeutics, Child & Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY

Purpose Adverse drug reactions (ADRs) rank as the fifth leading cause of death in the western world. The nature and scope of these ADRs in children are not predictable based on post market surveillance reports that rely heavily on adult drug experience. The genotype-specific approaches to therapy in childhood (GATC) national ADR network was established to identify specific ADRs and to improve drug safety through identification of predictive genomic biomarkers of drug risk.

Methods GATC set out to establish a national network of trained surveillance clinicians in pediatric hospitals across Canada. Surveillance clinicians identified, enrolled, and collected clinical data and biological samples from ADR cases and controls. Surveillance was targeted to three ADRs: anthracycline-induced cardiotoxicity, cisplatin-induced hearing impairment, and codeine-induced mortality in breastfed infants.

Results The initial surveillance site was established in September 2005, with 10 sites fully operational by 2008. In 3 years, GATC enrolled 1836 ADR cases and 13188 controls. Target numbers were achieved for anthracycline-induced cardiotoxicity. Modified target numbers were nearly attained for cisplatin-induced hearing impairment. Codeine-induced infant mortality in a breastfed infant was discovered by GATC investigators. A case-control study was subsequently conducted.

Conclusion GATC has demonstrated a model of active and targeted surveillance that builds an important step toward the goal of personalized medicine for children. Effective communication, site-specific solutions and long-term sustainability across the network are critical to maintain participation and productivity. GATC may provide a framework of ADR surveillance that can be adapted by other countries and healthcare systems. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS—adverse drug reaction; pharmacogenomics; surveillance; children

Received 19 December 2008; Revised 1 April 2009; Accepted 14 April 2009
The GATC Project

**Hypothesis**
Genetic polymorphisms in drug biotransformation genes underlie a significant portion of concentration-dependent ADRs in children.

**Goal**
To develop genotype-based dosing guidelines to predict safety and avoid severe ADRs in children.
Canadian Pharmacogenomics Network for Drug Safety

CPNDS Centre
- ADR Surveillance Core
- Genotyping Core
- Sequencing Core
- Health Economics Core
- Knowledge Translation
- Clinical Diagnostic Lab

Added 5 new sites in 2009 (Montréal, St. John’s, Hamilton, Edmonton, Kingston)
GATC Clinical Surveillance Network

- Identify children with ADRs
- Identify ‘matched’ children on same medications, without ADRs
- Look for genetic variation in key drug ADME enzymes
  - Informs new-drug development
- Develop new dosing guidelines
- *Bedside-benchtop-bedside science*
ADR Surveillance-Sample Collection

- Surveillance sites within hospitals/centres
  - Inpatient wards
  - Outpatient clinics
  - Emergency departments

- Whenever possible, DNA samples is collected from biological parents of ADR patients
GATC Overview

Adverse Drug Reaction

DNA collected

DNA genotyped

Integrate clinical & genotype data in association analyses

1900 SNPs in 200 analyses

Candidate genes

Detailed Clinical Data Collected
Recruitment of ADR Cases and Drug-Matched Controls

- ADR Cases and Controls
- Severe ADR Cases
- Drug-Matched Controls

March 2006 to March 2010

- 3,072 Severe ADR Cases
- 25,008 Drug-Matched Controls
We are all more than 99% genetically identical
Single Nucleotide Polymorphisms (SNP)

- Variations in DNA (frequency >1%)
- SNPs make up >90% of genetic variation
- When comparing 2 people:
  - 1 SNP occurs every 600-1200 bp
  - (= 5-10 million differences, ~99.9% identical)
- 14.7 Million known SNPs (January 2009)
- SNPs can alter the amino acid sequence of the encoded protein as well as alter RNA splicing and transcription
- New technology can test > 24 million SNPs per day
## ADME/Tox Genes SNP Arrays

### Gene Classification

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I Metabolizing Enzymes</td>
<td>CYP1A1, CYP2B6, ALDH2</td>
</tr>
<tr>
<td>Phase II Metabolizing Enzymes</td>
<td>UGT2B7, GSTM1, NAT1, COMT</td>
</tr>
<tr>
<td>Receptors / Drug Targets</td>
<td>VDR, PPARG, CETP</td>
</tr>
<tr>
<td>Transporters</td>
<td>ABCB1, ABCC1, ABCC2</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>HNF4A, STAT3, NR1I2</td>
</tr>
<tr>
<td>Immunity</td>
<td>HLA variants</td>
</tr>
<tr>
<td>Ion Channels</td>
<td>SCN5A, KCNH2, KCNQ1</td>
</tr>
<tr>
<td>Others</td>
<td>EPHX1, FMO1, PTGS1</td>
</tr>
</tbody>
</table>

### Versions:

**Initial:** 2k ADME SNP panel (220 genes)

**Current:** 4.6k ADME (300 genes) or 1.2M genome-wide scan

**Future:** 8k ADME & 2.5-5M+ arrays Genome Sequencing
Genotyping To Identify ADR-Associated Variants

DNA Purification Robot

2D Laser Etched Bar-coded Samples

Long Term Storage -80°C

New Illumina BeadXpress
1-384 Variants per sample

Illumina BeadStation
384-1,100,000 Variants per sample
Year 2000
Factory-size Sequencing Center with 350 DNA Sequencers

14 years to sequence the human genome
Cost: $2.7 billion

Year 2010
1 Bench Top High-Throughput DNA Sequencer

1 week to sequence the human genome
Cost: $20,000
Initiated analyses of prioritized drugs and reactions:

1. Cisplatin-induced hearing loss
2. Anthracycline-induced cardiotoxicity
3. Codeine-induced infant mortality
4. Life-threatening skin reactions
5. Vincristine-induced neuropathy
Cisplatin

- Drug of choice for solid tumours including hepatoblastoma, ovarian, CNS, osteosarcoma, neuroblastoma, lung, bladder, head and neck tumors
- **1,000,000 new patients** receive cisplatin each year (N. America & Europe)

Cisplatin-Induced Deafness ADR

- Causes permanent hearing loss
- 10-38% of patients
- Increased frequency and severity in children
  - 28%-61% of children 5-14 develop severe hearing loss
  - 38%-62% of children <5 yrs old develop severe hearing loss (Li et al, 2004)
- B.C. Children’s Hospital: 37% of patients developed grade 3-4 deafness since 2005
162 pediatric patients with hepatoblastoma, brain tumor, germ cell tumors, neuroblastoma, osteosarcoma

Classification of Cisplatin ADR Cases and Controls

**Controls**

- **Grade 0: Normal Hearing**
  Hearing threshold of 20 dB or less (within normal range) at all frequencies

**ADR Cases**

- **Grade 1 Hearing Loss: Mild High Freq. Loss**
  Minimum hearing threshold of 20-25 dB (4000 Hz and above)

- **Grade 2 Hearing Loss: Moderate High Freq. Loss**
  May require speech therapy or intervention with hearing aid
  Minimum hearing threshold of 25-39 dB (4000 Hz and above)

- **Grade 3 Hearing Loss: Severe Hearing Loss**
  Requires intervention with hearing aid
  Minimum hearing threshold of 25-39 dB (2000 Hz and above)

- **Grade 4 Hearing Loss: Deafness**
  Requires intervention with cochlear implant
  Minimum hearing threshold of 40dB or more (1000Hz and above)
Multistage Approach

Stage 1: Discovery
- N = 53 Vancouver
- P < 0.01
- Genotype full set of SNPs in relatively small population at liberal p value

Stage 2: Replication
- N = 109 Canada-wide
- P < 0.005
- Screen second, larger population at more stringent p value

Joel Hirschhorn & Mark Daly, Nature Reviews, 2006
Identified Genetic Variants Associated with Cisplatin-Induced Deafness

### Combined Discovery + Replication (n = 162)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Cases</th>
<th>Controls</th>
<th>O.R.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT</td>
<td>Intron</td>
<td>23.6%</td>
<td>1.8%</td>
<td>16.8</td>
<td>2.2 x 10(-4)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMT</td>
<td>Intron</td>
<td>29.2%</td>
<td>7.1%</td>
<td>5.5</td>
<td>1.8 x 10(-4)*</td>
</tr>
</tbody>
</table>

1. Loss of **TPMT**: Increased Cisplatin Toxicity
   - Cisplatin binds purines → DNA cross-linking → Cell death
   - TPMT normally inactivates purine-compounds (e.g. cisplatin)

2. Loss of **COMT**: Increased Cisplatin Toxicity
   - COMT and TPMT both use ‘S-adenosyl-L-methionine’ (SAM) substrate
   - Accumulation of SAM substrate is toxic in the presence of cisplatin
DNA Sequencing Identified Loss-of-Function TPMT Variants Associated with Cisplatin-Ototoxicity

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Combined (n = 162)</th>
<th>Cases</th>
<th>Controls</th>
<th>O.R.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT</td>
<td>Non-synon, loss of activity</td>
<td></td>
<td>23.6%</td>
<td>1.8%</td>
<td>16.9</td>
<td>2.2 x 10(^{-4})*</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>Intron, tag SNP</td>
<td></td>
<td>16.0%</td>
<td>1.8%</td>
<td>10.9</td>
<td>0.0017</td>
</tr>
<tr>
<td>TPMT*3B</td>
<td>Non-synon, loss of activity</td>
<td></td>
<td>14.1%</td>
<td>0%</td>
<td>18.0</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

TPMT*3B and *3C are the two key TPMT low activity variants responsible for **TPMT enzyme deficiency**
COMT: Catechol-O-Methyltransferase

*Loss of function* linked with deafness in mice/humans

A catechol-O-methyltransferase that is essential for auditory function in mice and humans


Departments of *Genetics and Cell Biology, Institute for Childhood and Neglected Diseases, and **Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA 92037, Department of Otolaryngology and the interdisciplinary Ph.D. Genetic Program, University of Iowa, 300 Hawkins Drive, Iowa City, IA 52242, *School of Medicine, University of California, San Diego, La Jolla, CA 92037, *Genomics Institute of the Novartis Research Foundation, San Diego, CA 92121, and **Genetic Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

Mutations of *LRTOMT*, a fusion gene with alternative reading frames, cause nonsyndromic deafness in humans

Zubair M Ahmed1,13, Saber Masmoudi2,13, Ersan Kalay3,5,13, Inna A Belyantseva1, Mohamed Ali Mosrat2, Rob W J Collin3,4, Saima Riazuddin1, Mounira Hmani-Aifa2, Hanka Venselaar5, Mayya N Kaur1, Abdelaziz Tili2, Bert van der Zwaag7, Shahid Y Khan6, LeilA Ayadi2, S Amer Riazuddin8, Robert J Morell1, Andrew J Griffith9, Ilhem Charfedine10, Refik Çaylan11, Jaap Oostrik4, Ahmet Karaguzel12, Abdelmonem Ghorbel10, Sheikh Riazuddin8, Thomas B Friedman1, Hammadi Ayadi2 & Hannie Kremer4,12

Mutation in *TRMU* Related to Transfer RNA Modification Modulates the Phenotypic Expression of the Deafness-Associated Mitochondrial 12S Ribosomal RNA Mutations

Min-Xin Guan, Qingfeng Yan, Xiaoming Li, Yelena Bykhovskaya, Jaime Gallo-Teran, Petr Hajek, Noriko Umeda, Hui Zhao, Gema Garrido, Emiette Mengeshia, Tsutomu Suzuki, Ignacio del Castillo, Jennifer Lynne Peters, Ronghua Li, Yaping Qian, Xinjian Wang, Ester Ballana, Mordchai Shohat, Jianxin Lu, Xavier Estivill, Kimisuna Watanabe, and Nathan Fischel-Ghodsian
Combining Top 2 SNPs in *TPMT* and *COMT* Identifies 48% of Cisplatin Ototoxicity Cases with High Specificity

<table>
<thead>
<tr>
<th>Deaf Cases</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined TPMT/COMT</td>
<td>48.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity: Specificity:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>48.1%</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PPV: NPV:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>92.7%</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>12.1</strong></td>
<td><strong>3.4x10^{-8}</strong></td>
</tr>
</tbody>
</table>

![Predicted Ototoxicity](graph.png)

- **Patients (%)**
  - Moderate to Severe Hearing Loss
  - Normal Hearing Loss
Increasing Numbers of Risk Alleles: Increased Severity, Frequency, and Earlier Onset of Hearing Loss

![Graph showing the relationship between days post cisplatin treatment and the percentage of patients with normal hearing. The graph indicates a decrease in patients with normal hearing as the number of risk alleles increases. The legend includes lines for 0 risk alleles (black), 1 risk allele (green), 2 risk alleles (blue), and 3+ risk alleles (red). The p-value for the comparison is P < 0.0001.]

### Table: Number of TPMT and/or COMT risk alleles

<table>
<thead>
<tr>
<th>Number of TPMT and/or COMT risk alleles</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ototoxicity patients (grade 1+)</td>
<td>56 (51.9%)</td>
<td>41 (93.2%)</td>
<td>12 (92.3%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Normal hearing controls</td>
<td>52 (48.1%)</td>
<td>3 (6.8%)</td>
<td>1 (7.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ototoxicity grade (mean ± s.e.m.)</td>
<td>1.53 ± 0.53</td>
<td>2.57 ± 0.14</td>
<td>2.62 ± 0.21</td>
<td>3.00 ± 0</td>
</tr>
</tbody>
</table>
Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy
### What Next?
**Patient Predicted to be at High Risk for Cisplatin-Induced Ototoxicity**

What is done now without a predictive test:

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Treatment</th>
<th>Ototoxicity</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteosarcoma</td>
<td>Doxorubicin &amp; cisplatin</td>
<td>Grade 2</td>
<td>Reduce cisplatin 50%</td>
</tr>
<tr>
<td>CNS tumors</td>
<td>Cisplatin, Etoposide, + Vincristine</td>
<td>Grade 3+</td>
<td>Discontinue cisplatin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 2</td>
<td>Reduce cisplatin 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grade 3+</td>
<td>Discontinue cisplatin</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Doxorubicin + cisplatin</td>
<td>Grade 3+</td>
<td>Discontinue cisplatin</td>
</tr>
</tbody>
</table>
What Next?
Patient Predicted to be at High Risk for Cisplatin-Induced Ototoxicity

Predictive testing:
- Alternative drug
- Increase monitoring in high risk patients e.g. patients in rural centres
- Experimental Protective Strategies to prevent cisplatin-ototoxicity
  - Sodium Thiosulfate
  - N-acetylcysteine D-methionine
  - Glutathione ethyl ester
Requirements for Entry of a Pharmacogenetic Diagnostic Test into Clinical Practice

- Codeine-Induced Infant Mortality
- Anthracycline-Cardiotoxicity
- Cisplatin Hearing Loss
- Severe skin reactions, Vincristine neuropathy, Ifosfamide nephrotoxicity, Methotrexate N/V...
Incorporation of validated ADR biomarker into diagnostic chip

Patient to receive drug “X”

ADR Screen Report
Drug X: 10-fold increased risk of reaction
Drug Y: 5-fold increased risk
Drug Z: 20-fold increased risk
## Overview of Progress

<table>
<thead>
<tr>
<th>ADR (Phenotype)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statin-induced severe muscle myopathy (CK &lt; 10x ULN)</td>
<td>Genetic variants in SLCO1B1 associated with severe muscle myopathy (Brunham et al, Submitted)</td>
</tr>
<tr>
<td>Cisplatin-induced deafness (CTCAE Grade 2+ hearing loss)</td>
<td>Genetic variants in TPMT and COMT associated with serious hearing loss (Ross et al, <em>Nature Genetics</em>, 2009)</td>
</tr>
<tr>
<td>Anthracycline-induced severe cardiotoxicity (CTCAE Grade 2+ cardiotoxicity)</td>
<td>Genetic variants in 7 genes associated with cardiotoxicity (Visscher et al, <em>In Submission</em>, 2010)</td>
</tr>
<tr>
<td>Codeine-induced CNS depression</td>
<td>Validation of genetic variants in CYP2D6 and UGT2B7 associated with codeine-induced CNS depression (Madadi et al, <em>Clin. Phar &amp; Ther</em>, 2009 and 2010)</td>
</tr>
</tbody>
</table>
Canadian Pharmacogenomics Network for Drug Safety

Bruce Carleton, UBC/CFRI/BCCH
Michael Hayden, UBC/CMMT/CFRI
Michael Phillips, Montréal
Colin Ross, UBC/CFRI/CMMT
Steve Leeder, Children’s Mercy Hospital
Gideon Koren, Univ Toronto
Stuart MacLeod, UBC/CFRI/PHSA
Michael Rieder, UWO
David Freeman, UWO
Marie-Pierre Dubé, Montréal
Wyeth Wasserman, UBC/CFRI/CMMT
Craig Mitton, UBC
Adrian Levy, Dalhousie University
Paul Rogers, BC Children’s/CFRI/UBC
Rod Rassekh, BC Children’s/CFRI/UBC

ADR Surveillance Investigators and Surveillors
Vancouver: Claudette Hildebrand, Tina Wong, Reza Ghannadan
Calgary: David Johnson, Linda Verbeek, Rick Kaczowka
Edmonton: Corrine Sikora
Winnipeg: Kevin Hall and Shanna Chan
Toronto: Gideon Koren, Shinya Ito, Miho Inoue
London: Michael Rieder and Becky Malkin
Ottawa: Herpreet Mankoo, Regis Vaillancourt
Hamilton: Amy Cranston
Montreal: Jean-Francois Bussières, Denis Lebel, Pierre Barret
Halifax: Margaret Murray, Darlene Boliver and Carol-anne Osborne