Genomic Approaches to Adverse Drug Reactions in Children

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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

Case 2

BASELINE STUDIES
Case 1

Case 2

MIDPOINT OF THERAPY
(AFTER 2 CYCLES OF CISPLATIN)
CURRENT STUDIES

Case 1

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

- 4-6\textsuperscript{th} leading cause of death in the USA\textsuperscript{1}
- Health care costs: $137-177 billion annually (USA)\textsuperscript{2-3}
- Cause 7\% of all hospital admissions\textsuperscript{4}
- Cause serious reactions in over 2,000,000 hospitalized patients (6.7\%) each year in the USA\textsuperscript{1}
- Cause fatal reactions in over 100,000 hospitalized patients each year in the USA\textsuperscript{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

1. Lazarou et al, JAMA, 1998
4. Pirmohamed et al, BMJ, 2004
5. MjoÈrndal et al, EACPT3, 1999
6. Moore et al., 2007
Pharmacogenomics

- Avoid adverse drug reactions
- Maximize drug efficacy for individual patients

All Patients with Same Diagnosis

- 10% risk of adverse reaction

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
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† ‡

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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

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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.
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
DNA genotyped 1900 SNPs in 200 Candidate genes

Integrate clinical & genotype data in association analyses

Detailed Clinical Data Collected

DNA collected

Adverse Drug Reaction
Recruitment of ADR Cases and Drug-Matched Controls

ADR Cases and Controls

- March 2006
- March 2007
- March 2008
- March 2009
- March 2010

25,008 Drug-Matched Controls
3,072 Severe ADR Cases
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
<table>
<thead>
<tr>
<th>Gene Classification</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
Cisplatin-ADR Patient Recruitment

- 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

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

Stage 1: Discovery

N = 53 Vancouver

Genotype full set of SNPs in relatively small population at liberal $p$ value

$P < 0.01$

Stage 2: Replication

N = 109 Canada-wide

Screen second, larger population at more stringent $p$ value

$P < 0.005$

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>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>
</tr>
</thead>
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<tr>
<td>TPMT</td>
<td>Non-synon, loss of activity</td>
<td>23.6% 1.8% 16.9 2.2 x 10(^{-4})*</td>
</tr>
<tr>
<td>TPMT(^*3C)</td>
<td>Intron, tag SNP</td>
<td>16.0% 1.8% 10.9 0.0017</td>
</tr>
<tr>
<td>TPMT(^*3B)</td>
<td>Non-synon, loss of activity</td>
<td>14.1% 0% 18.0 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

Zubair M Ahmed1,13, Saber Masmoudi2,13, Ersan Kalay3–5,13, Inna A Belyantseva1, Mohamed Ali Mosrati7, Rob W J Collin3,4, Saima Riazuddin1, Mounira Hmani-Alia4, Hanka Venselaar5, Mayya N Kaur1, Abdelaziz Tlii2, Bert van der Zwaag7, Shahid Y Khan8, Leila Ayadi2, S Amer Riazuddin8, Robert J Morell1, Andrew J Griffith9, Ilhem Charfeddine10, Refik Cavlan11, Jaap Oostrik4, Ahmet Karaguzel1, Abdelmonem Ghorbel10, Sheikh Riazuddin8, Thomas B Friedman1, Hammad Ayadi2 & Hannie Kremer4,12

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

Min-Xin Guan, Qingfeng Yan, Xiaoming Li, Yelena Bykhovskaya, Jaime Gallo-Teran, Petr Hajek, Noriko Umeda, Hui Zhao, Gema Garrido, Emabet Mengesh, Tsutomu Suzuki, Ignacio del Castillo, Jennifer Lynne Peters, Ronghua Li, Yaping Qian, Xinjian Wang, Este Ballana, Mordechai Shohat, Jianxin Lu, Xavier Estivill, Kimitsuna Watanabe, and Nathan Fischel-Ghodsian

Methyltransferases
Combining Top 2 SNPs in *TPMT* and *COMT* Identifies 48% of Cisplatin Ototoxicity Cases with High Specificity

<table>
<thead>
<tr>
<th></th>
<th>Deaf Cases</th>
<th>Normal Hearing Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Combined TPMT/COMT</strong></td>
<td>48.1%</td>
<td>7.1%</td>
</tr>
</tbody>
</table>

**Odds ratio** 12.1, **p-value** $3.4 \times 10^{-8}$

**Sensitivity:** 48.1%, **Specificity:** 92.9%

**PPV:** 92.7%, **NPV:** 48.6%
Increasing Numbers of Risk Alleles: Increased Severity, Frequency, and Earlier Onset of Hearing Loss

- 0 risk alleles
- 1 risk allele
- 2 risk alleles
- 3+ 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>

P < 0.0001
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></td>
<td></td>
<td>Grade 3+</td>
<td>Discontinue cisplatin</td>
</tr>
<tr>
<td>CNS tumors</td>
<td>Cisplatin, Etoposide, + Vincristine</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><strong>Statin-induced severe muscle myopathy</strong> <em>(CK &lt; 10x ULN)</em></td>
<td>Genetic variants in SLCO1B1 associated with severe muscle myopathy (Brunham et al, <em>Submitted</em>)</td>
</tr>
<tr>
<td><strong>Cisplatin-induced deafness</strong> <em>(CTCAE Grade 2+ hearing loss)</em></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><strong>Anthraclycline-induced severe cardiotoxicity</strong> <em>(CTCAE Grade 2+ cardiotoxicity)</em></td>
<td>Genetic variants in 7 genes associated with cardiotoxicity (Visscher et al, <em>In Submission</em>, 2010)</td>
</tr>
<tr>
<td><strong>Codeine-induced mortality</strong> in infants and neonates</td>
<td>Genetic variants in CYP2D6 and UGT2B7 associated with codeine-induced CNS depression (Koren et al, <em>Lancet</em>, 2006; Czikowski et al, <em>NEJM</em>, 2009)</td>
</tr>
<tr>
<td><strong>Codeine-induced CNS depression</strong></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

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