Pharmacogenetics of Severe Adverse Drug Reaction

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Paradox of Modern Drug Development

1. Clinical trials provide evidence of efficacy and safety at usual doses in *populations*

\[ \text{Safe & Effective} \]

2. Physicians treat *individual* patients who can vary widely in their response to drug therapy

\[ \text{Safe & Effective} \quad \text{No Response} \quad \text{Adverse Drug Reaction} \]
Adverse Drug Reactions

- 5th leading cause of death in the USA
  - Over 100,000 fatal ADRs in hospitalized patients each year
  - Over 2,000,000 serious ADRs in hospitalized patients (6.7%)/yr
- ADRs cause 7% of all hospital admissions (UK)
- ADR Health care costs: $78-177 billion annually (USA)
  - Exceeds the annual cost of medications
- ADRs cause an average 2 day increase in hospital stays
- 95% of all ADRs are unreported
“12% of patients rushed to Vancouver General Hospital have adverse reactions to medications.”


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50% of newly approved therapeutic health products have **serious ADRs**, discovered only after the product is on the market

(Health Canada, 2007)
ADRs in Children
Increased Risk of Severe ADRs in Children

- 11-15% of hospitalized children have an ADR\textsuperscript{1-3}
- ADRs cause 22% of admissions in pediatric cancer patients\textsuperscript{4}
- 30% of ADRs in hospitalized children are severe causing long-term disability or death\textsuperscript{4}
- 26,000 children die each year from ADRs in USA\textsuperscript{5}
- Gross lack of knowledge about ADRs in children

3. Takata et al, Pediatrics, 2008
5. Lazarou et al, JAMA, 1998
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
Factors Contributing to Variability in Drug Response

- Genetic Factors: 20-95%
- Ethnicity
- Diet
- Compliance
- Concomitant Disease
- Concomitant Drugs
- Age
- Weight
- Gender

Patient genotype is currently an unknown factor in the prescribing of medicines.
How Can The Causes of Variability be Unraveled?
The Canadian Pharmacogenomics Network for Drug Safety

**Hypothesis**
- Genetic polymorphisms in drug metabolism 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.
Goal is to predict safety and avoid potential complications, **not** to make effective drugs difficult to obtain for patients.
ADR Surveillance
Over 95% of ADRs are not reported

2 studies identified patients being treated for drug-induced T.E.N. in burn units

Q: What % of these ADRs were reported?


2.5% ADR Reporting Rzany et al., J Clin Epidemiol 1996;49(7):769-73
Recruitment of ADR Cases and Drug-Matched Controls

![Graph showing recruitment of ADR cases and drug-matched controls over time. The x-axis represents the date from September 2005 to March 2009, labeled as quarterly intervals: Sep, Dec, Mar, Jun. The y-axis represents the number of participants enrolled, ranging from 0 to 18,000. The graph shows two lines: one for severe ADR cases and another for drug-matched controls. The severe ADR cases start with 1,000 participants in September 2005 and reach 2,283 by March 2009. The drug-matched controls start with 1,066 participants in September 2005 and reach 16,842 by March 2009. Each quarter shows an increase in participants enrolled.](image)
Genomic Analyses
Association Study

Patients with Disease

Control Unaffected Patients
Association Study

Patients with Disease

Control Unaffected Patients

Odds Ratio = 16
P value = 0.02
Genome Canada’s GATC Project supported the acquisition of the Illumina 500GX platform at the CMMT.

Centre for Molecular Medicine & Therapeutics
Vancouver, B.C.
ADME/Tox Genes SNP Arrays

<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>

**Current**: 3072 SNP array

**Other options**: 6144 to 1.1 million SNP arrays

1536 HapMap derived haplotype tag SNPs

1536 Altered enzyme activity common non-synonymous, literature validated rare non-synonymous, synonymous coding SNPs
SNP Genotyping

DNA Purification Robots

2D Laser Etched Bar-coded Samples

Illumina BeadStation
384-1.2 million test per sample

Illumina BeadXpress
1-384 tests per sample

Long Term Storage -80°C

DNA (blood, saliva, buccal)
Illumina SNP Genotyping

20 million beads on one slide
Illumina SNP Genotyping

DNA target capture probe
affixed to bead:
Complementary to SNP region (50 bp)
Illumina SNP Genotyping

Complementary DNA from patient DNA bound to probe

Bead
Slide
Illumina SNP Genotyping

Single-nucleotide extension (biochemical reaction)

Bead
Slide

[G]

[G - C]
Illumina SNP Genotyping

Single-nucleotide product fluorescently labeled

Individual with “T” genotype at this site

[G]

[G -C]

[T -A]

Bead

Slide
Illumina SNP Genotyping

1.2M Chip (1.2 million SNPs)
2 Samples/Chip

TT/TT  TT/GG  GG/GG
Examples of SNP Genotyping Formats

1. Custom SNP Panels
   - 1 to 200,000 SNPs/assay

2. Human Genome-wide SNP panels
   - 300,000 SNPs Genome-wide
     - 12 samples/chip
     - Highly cost-effective
   - 660,000 SNPs Genome-wide
   - 1.2 Million SNPs Genome wide
Raw Fluorescence Intensity Data

SNP #1 (480 samples)

Green Signal

Yellow Signal

Intermediate Signal

Red Signal

Genotype Text Output

SNP 1  AA
SNP 2  TT
SNP 3  GG
SNP 4  GC
Raw SNP Data (n = 480)
SNP assay conversion rates for tag SNPs: 90-96%

High Genotype Call Rates: >99%

High Reproducibility: >99.99%
- 16 miscalls out of 178,860 genotype calls (58 patient DNA replicates)
- 0 miscalls out of 50,688 genotype calls (16 control DNA replicates)
30 replicate assays for each SNP
Illumina BeadXpress (Veracode) SNP Genotyping

- 384 samples x 384 SNPs

- SNP Conversion Rate: **95.05%** (368/384)
  (# of SNPs that could be assayed)

- Average Call Rate: **99.32%** (min. 93%, max. 99.7%)
Illumina BeadXpress (Veracode) SNP Genotyping

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Average SNP: GenCall Score = 0.86

Cartesian View

Polar View
Illumina BeadXpress (Veracode) SNP Genotyping

- 384 samples x 384 SNPs

- SNP Conversion Rate: 95.05% (368/384) (# of SNPs that could be assayed)

- Average Call Rate: 99.32% (min. 93%, max. 99.7%)

Best SNP: GenCall Score = 0.969
Illumina BeadXpress (Veracode) SNP Genotyping

- 384 samples x 384 SNPs
- SNP Conversion Rate: 95.05% (368/384) (# of SNPs that could be assayed)
- Average Call Rate: 99.32% (min. 93%, max. 99.7%)

Worst SNP: GenCall Score = < 0.30

Cartesian View Polar View
CPNDS Priority ADR Targets

*In Progress:*
- Codeine-induced infant mortality
- Cisplatin-induced deafness
- Anthracycline-induced cardiotoxicity
- Life-threatening skin reactions
- Vincristine-induced neuropathy
- Statin-induced muscle damage
- Interferon-β toxicity
- Warfarin-induced bleeding/thrombosis
Codeine
The American Academy of Pediatrics and major authoritative texts list codeine as compatible with breastfeeding

– Briggs et al., 2005; Pediatrics, 2001
Case Report
- A new mother was given Tylenol #3 for obstetric pain relief
  - Given a standard dose (60 mg every 12 hours)
- Mother complained of significant drowsiness
  - Codeine dose cut in half (30 mg every 12 hours)
- Infant showed poor feeding
- Infant died on day 13 due to respiratory failure

Follow-up Analysis:
- Maternal milk from last day of the baby’s life contained morphine at 10-20x higher levels than expected (87 ng/ml)
- Infant’s blood contained lethal levels of morphine (70 ng/ml)
Identified genetic variants associated with a lethal adverse reaction to codeine in newborns

Mother’s Genotype:
- CYP2D6 gene duplication
- UGT2B7*2/*2

Outcome:
- Accumulation of morphine in breast milk (10-20x more than normal)
- Breast milk fed to infant
- Infant died at 13 days of age
- Lethal levels of morphine accumulated in the infant causing CNS depression, respiratory failure, and death
Pharmacogenetics of Neonatal Opioid Toxicity Following Maternal Use of Codeine During Breastfeeding: A Case–Control Study

P Madadi\textsuperscript{1,2}, CJD Ross\textsuperscript{3}, MR Hayden\textsuperscript{3}, BC Carleton\textsuperscript{4}, A Gaedigk\textsuperscript{5}, JS Leeder\textsuperscript{5} and G Koren\textsuperscript{1,2,6}

A large number of women receive codeine for obstetric pain while breastfeeding. Following a case of fatal opioid poisoning in a breastfed neonate whose codeine prescribed mother was a CYP2D6 ultrarapid metabolizer (UM), we examined characteristics of mothers and infants with or without signs or central nervous system (CNS) depression following codeine exposure while breastfeeding in a case–control study. Mothers of symptomatic infants (n = 17) consumed a mean 59\% higher codeine dose than mothers of asymptomatic infants (n = 55) (1.62 (0.79) mg/kg/day vs. 1.02 (0.54) mg/kg/day; \(P = 0.004\)). There was 71\% concordance between maternal and neonatal CNS depression. Two mothers whose infants exhibited severe neonatal toxicity were CYP2D6 UMss and of the UGT2B7*2/*2 genotype. There may be a dose–response relationship between maternal codeine use and neonatal toxicity, and strong concordance between maternal infant CNS depressive symptoms. Breastfed infants of mothers who are CYP2D6 UMss combined with the UGT2B7*2/*2 are at increased risk of potentially life-threatening CNS depression.
FDA drug label change and public health advisories

Estimated 1846 newborn infants are at risk for this codeine ADR each year in Canada

(340,000 births, 73% breastfed, 52% mothers receive codeine post-childbirth, 1.4% risk genotype)
Additional Cases of Infant Toxicity from Codeine from Literature

- 35 reports of breastfeeding infants with ADRs to codeine, including:
  - Unexplained severe drowsiness
  - Apnea
  - Bradycardia
  - Cyanosis
Currently Performing Randomized Controlled Trial

Prospective study to test the assessment of the benefit of a diagnostic test to prevent codeine ADRs in infants

Study Design:

- 600 women booked for elective C-section

  - Prospective Screening for CYP2D6-UM Predictive Variants
    - N = 300
    - + Test: N = 12 (est.)
    - - Test: N = 288 (est.)

  - Control Group (standard care) N = 300

  - Receive Ibuprofen or Naproxen post-partum
    - Standard Care: Receive codeine & monitoring for CNS depr. ADR
    - Standard Care: Receive codeine & monitoring for CNS depression ADR

  - Compare Outcomes
    - Adverse events
    - Adequate pain relief?
    - Cost of ADRs
    - Hospitalization
    - Cost of care
    - Cost of Screening
    - Outcome of therapy/survival
    - Treatment Compliance
    - Validity of Diagnostic Test

  - Retrospective genetic screening for CYP2D6-UM
ADRs in Chemotherapy
Cancer Survival has Improved, but Survivors often Left with Lifelong Consequences of Severe ADRs

82% of children beat cancer

Survivors are often left with lifelong health consequences
By KRISTEN THOMPSON
April 10, 2008 02:24

More children are surviving cancer than ever before, according to statistics released yesterday by the Canadian Cancer Society. But experts are finding that survival often carries lifelong health consequences.
Seven-year-old Casey Wright, from Maple Ridge, is among the 82 per cent of children diagnosed with cancer who survive thanks to progress in treatments — an 11 per cent increase in the past 15 years.
But he also represents the two-thirds of survivors who have to live with chronic or late-occurring health effects.

Casey Wright, 7, hugs his sister Jemma, 9, during lunch hour yesterday at Maple Ridge elementary as their mother, Kim, discusses his recovery from a malignant brain tumour.
Pediatric Oncology:

- 1 in 750 young adults are survivors of childhood cancer
- 75% of cancer survivors suffered at least 1 ADR
- 40% of cancer survivors have had a severe ADR (life-threatening, or disabling)
- 25% of cancer survivors suffer 5 or more ADRs

Geenan et al, *JAMA*, 2007
Cisplatin-Induced Deafness
Cisplatin

- A highly effective anti-tumor agent
- Treatment of solid tumours including ovarian, lung, bladder, head and neck
- In children: treatment of CNS tumors, hepatoblastoma, neuroblastoma, osteosarcoma
- >1,000,000 patients receive each year (N. America & Europe)
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

OVERALL: Cases sound similar
- Same tumor, treatment, and cure outcomes
Case 2 Suffered Severe Hearing Loss

Case 1

Normal Hearing Audiogram

Case 2

Severe Hearing Loss
Cisplatin-Induced Deafness

- Causes permanent hearing loss
  - Bilateral, hair cell degeneration in cochlea
  - Initially high freq. loss (cells with higher metabolic activity)

- 10-38% of adult patients affected

- 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
Cisplatin-ADR Patient Recruitment

162 pediatric patients with hepatoblastoma, brain tumor, germ cell tumours, 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

n = 56

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

May require speech therapy or intervention with hearing aid

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)

ADR Cases

n = 106
Multistage Approach

Stage 1: Discovery
N = 55 Vancouver
Genotype full set of SNPs in relatively small population at liberal $p$ value

Stage 2: Replication
N = 107 Canada-wide
Screen second, larger population at more stringent $p$ value

$P < 0.005$

$P < 0.01$

Joel Hirschhorn & Mark Daly, *Nature Reviews*, 2006
**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>
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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
In the Future

Pharmacogenomics could have profound impact in medicine

Advances in technology opening the doors to understanding the genetic factors of ADRs

- Whole Genome Sequencing
- Routine genotyping of millions of SNP variants

Lower health care costs:

- ADRs now exceed the cost of medications in USA/Canada

Improved safety

- Safer and More Effective Treatments
Canadian PGx Network for Drug Safety

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