Pharmacogenomics and the Transformation of Practice

Dr. Bruce Carleton
Professor of Pediatrics, Pharmaceutical Sciences, Population & Public Health, Medical Genetics, University of British Columbia
Director, Pharmaceutical Outcomes Programme, BC Children's Hospital
Senior Clinician Scientist, Child & Family Research Institute
Vancouver, CANADA

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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

1. Lazarou et al, JAMA, 1998
4. Pirmohamed et al, BMJ, 2004
5. MjoÈrndal et al, EACPT3, 1999
6. Moore et al., 2007
Paradox of Modern Drug Development

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

\[ \text{Efficacious & Safe} + \text{Efficacious & Safe} = \text{Efficacious & Safe} \]

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

\[ \text{No Response} + \text{Efficacious & Safe} = \text{Efficacious & Safe} \]
\[ \text{Adverse Drug Reaction} \]

Factors Contributing to Variability in Drug Response

Genetic Factors

Patient genotype is currently an unknown factor in the prescribing of medicines

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 (25%): treat with alternative drug or dose
- Low risk of ADR (25%): 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

The CPNDS 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.

CPNDS Clinical Surveillance Network

- Identify children with ADRs
- Identify ‘matched’ children on same medications, without ADRs
- Whenever possible, DNA samples is collected from biological parents of ADR patients
- Look for genetic variation in key drug ADME enzymes
  - Informs new-drug development
- Develop new dosing guidelines
  - Bedside-benchtop-bedside science
1. Identify children with ADRs & matched controls
2. Collect DNA samples (blood/saliva)
3. Detailed patient clinical characterization
4. Screen genetic variants
5. Replication

CPNDS Biomarker Discovery Strategy

Recruitment of ADR cases and drug-matched controls in Canada

Severe ADR case reports

Number of ADR Reports

Drug-matched controls

Number of Control Reports

>7,896 ADR case reports

>70,023 drug-matched controls

Could take 4-5 hours, or up to 4-5 days to complete clinical characterizations
We are 99.9% 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

Gene Classification
Examples
Phase I Metabolizing Enzymes: CYP1A1, CYP2B6, ALDH2
Phase II Metabolizing Enzymes: UGT1A1, GSTM1, NAT1, COMT
Receptors / Drug Targets: VDR, PPARG, CETP
Transporters: ABCB1, ABCC1, ABCC2
Transcription factors: HNF4A, STAT3, NR1I2
Immunity: MHC variants
Ion Channels: SCN5A, KCNH2, KCNQ1
Others: EPHX1, FMO1, PTGS1

ADME/Tox Genes SNP Arrays

Versions:
Initial: 2k ADME SNP panel (220 genes)
Phase II: 4.6k ADME (300 genes) or 1.2M genome-wide scan
Current: 10k ADME & 2.5M+ arrays Genome Sequencing

Codeine-Induced Infant Mortality

Initial Case Report:
A new mother was given a standard dose of Tylenol #3 for obstetric pain relief
Complained of significant drowsiness and constipation: dose reduced by 50%
Infant showed poor feeding
Infant died on day 13 due to respiratory failure

Follow-up Analysis:
Infant’s blood contained lethal levels of morphine (70 ng/ml)
Coroner brings case to GATC
Maternal milk contained 87 ng/ml of morphine (10-20x expected)
Identified genetic variants associated with lethal reaction to codeine in newborns

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

Outcome:
- Accumulation of morphine in breast milk
- Breast milk fed to infant
- Accumulation of morphine in infant caused CNS depression, respiratory failure, and death

Prior to Our Work

The American Academy of Pediatrics and “Drugs in Pregnancy in Lactation”, the major reference guide to fetal and neonatal risk, list codeine as compatible with breastfeeding

- Briggs et al., 2005; Pediatrics, 2001

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

(340,000 births, 73% breastfed, 52% mothers receive codeine post-childbirth, 1.4% risk genotype)

August 20, 2009

- 2 year old boy
- Received tonsillectomy for sleep apnea
- Received standard codeine dose
- Died of respiratory depression
- High levels of morphine in blood (32 ng/ml)
- Boy carried CYP2D6 gene duplication
“...These cases demonstrate that analgesia with codeine or other opioids that use the CYP2D6 pathway after adenotonsillectomy may not be safe in young children with obstructive sleep apnea syndrome.”

**Anthracycline Case Report**

- A previously healthy 10-year-old presented with abdominal mass
- Biopsy confirmed neuroblastoma
- Patient began standard doxorubicin chemotherapy protocol
  - Cumulative dose: 300 mg/m²
Case Report

- A previously healthy 10-year-old child presented with neuroblastoma to B.C. Children’s Hospital
- Began doxorubicin chemotherapy
- Prior to last cycle of treatment, child became unwell during a routine CT scan at BC Children’s Hospital
  - Intubated and rushed to ICU
  - Developed serious cardiac dysfunction, virtually no cardiac output
  - Child placed on extracorporeal membrane oxygenation (ECMO) (heart-lung machine)
  - Child received a heart transplant
  - First transplanted heart rejected
  - Child received a second heart transplant
- Child is currently cancer remission

Anthracyclines

- Doxorubicin, Epirubicin, Daunorubicin, Idarubicin
- Administered to 70% all childhood cancer patients
- Adjuvant chemotherapy for 50-90% of breast cancer
- 22,000 patients/year in Canada
- At least 970,000 patients receive each year (N. America)

Highly effective

- Introduction of anthracyclines contributed to improved childhood cancer survival: from 30% in 1960s to >80% today

Anthracycline-Induced Cardiotoxicity

- Since 1967, recognized that anthracyclines can cause fatal cardiac toxicity (Tan et al., Cancer, 1967)
- 5-16% of patients suffer serious cardiomyopathy and heart failure
  - Toxicity can occur at doses <350 mg/m²
  - While some patients tolerate >1000 mg/m²
- May require intra-ventricular assist device or heart transplant
- Increased severity in children, especially less than 4 years old
- 72% mortality rate for severe cases (BC Cancer Agency 2010)
Anthracycline-induced Cardiotoxicity

- Most important risk factor is high cumulative dose
- However there is no absolute safe dose
- Large inter-individual variability suggests genetic susceptibility

Some individuals susceptible at any dose

Classification of Anthracycline-Cardiotoxicity

Controls n=266

- No cardiotoxicity, SF ≥30%, ≥5yr follow-up

ADR Cases n=78

- Grade 1 toxicity:
  - Shortening fraction 27-30% or
  - Resting ejection fraction 50-60%

- Grade 2 toxicity:
  - Moderate to severe cardiotoxicity
  - Shortening fraction <15% or Shortening fraction 15-26%
  - or resting ejection fraction 40-50%

- Grade 3 toxicity:
  - Symptomatic congestive heart failure
  - Shortening fraction <15% or
  - Resting ejection fraction <40%

- Grade 4 toxicity:
  - Congestive heart failure requiring heart transplant or ventricular assist device
  - Resting ejection fraction <20%

Further replication of SLC28A3 in a third independent Dutch cohort from Amsterdam

SLC28A3 Sodium-coupled nucleoside transporter 28A3

- Transporter of pyrimidines, purines, and anti-cancer drugs
- Demonstrated role in anthracycline transport (Nagasawa et al. Curr Drug Metab 2001)
- Expressed throughout body, including heart

Hypothesis:
SLC28A3 L461L is a protective allele:
- Reduced SLC28A3 expression
- Reduced anthracycline uptake
- Reduced generation of toxic metabolites
**Potential mechanism of SLC28A3**

- **SLC28A3**: Sodium-coupled nucleoside transporter broadly selective for pyrimidines and purines and anti-cancer drugs
  - Contributes to *influx of anthracyclines* in cancer cell lines (Nagasawa et al. *Curr Drug Metab* 2001)

- **Expressed throughout body, including heart**

- **L461L minor allele** (protective) associated with *reduced expression* in multiple cell lines:
  - $P$ adjusted = 0.0105

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**UGT1A6**

UDP glucuronosyltransferase 1A6

- Glucuronidates many different substrates:
  - Increases water-solubility and elimination of drug
  - Often directly reduces drug toxicity

- Val209Val synonymous risk variant tags the UGT1A6*4 haplotype:
  - *4 haplotype = S7A/L105L/R184S/V209V
  - *4 haplotype exhibits 30-100% reduced enzyme activity (Nagar et al., Pharmacogenetics, 2004; Krishnaswamy et al, J Phar Exp Ther, 2005)

**Hypothesis**: UGT1A6*4 is a risk haplotype

- Reduced UGT1A6 enzyme activity
- Reduced elimination and toxicity of anthracyclines or metabolites
Potential mechanism of UGT1A6

- UGT1A6: UDP glucuronosyltransferase 1A6 glucuronidates many different substrates
- Synonymous Val209Val variant tags the UGT1A6*4b haplotype: S7A/L105L/R184S/V209V
- UGT1A6*4 has 30-100% reduced enzyme activity
- Parent anthracycline compounds not glucuronidated, but metabolites undergo glucuronidation
- Altered glucuronidation may lead to accumulation of toxic anthracycline metabolites

Potential Clinical Options for Personalized Anthracycline Therapy

Depending on risk prediction, a clinician could take different actions:

- **Low Risk**
  - Echocardiogram follow-up as usual

- **Intermediate Risk**
  - Intensify echocardiogram follow-up
  - e.g., patients in rural centers often miss appointments

- **High Risk**
  - Alternative medication (e.g., mitoxantrone) or dose
  - Add cardioprotectant (e.g., dexrazoxane)
  - Start treatment with ACE inhibitors or beta-blockers to prevent further damage

SLC28A3 + UGT1A6 + Clinical Variables for Risk Prediction of Anthracycline Cardiotoxicity

ROC: AUC (SNPs + Clinical) = 0.76
**Advances in Genomics Technology**

**Year 2002**

- 12+ years to genotype 1 million variants throughout the genome
- Cost: $2.7 billion

**Year 2013**

- 2 days to genotype 1 million variants throughout the genome
- Cost: ~$250

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**Gaps in PGx Translation**

- Clear evidence of clinical utility
- Cost benefit analysis
  - How does pharmacogenetic testing contribute to sustainability of the health care system?
- Risk/benefit profiling for patients
- Limited supply of experts

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**Specific Objectives**

1. To develop pharmacogenetic clinical practice guidelines.
2. To implement an ADR prevention program at the Regional Cancer centres across B.C. beginning at B.C. Children's Hospital.
3. To determine how the PGx tests are perceived and utilized by patients, physicians, and families before and after administration of the test.
4. To evaluate the cost-effectiveness of the pharmacogenetic tests.
5. To educate and train clinicians on genomics and pharmacogenetics.
6. To lay the groundwork for the development of a B.C. pharmacogenetic ADR prevention program.
Implementing a ADR Prevention Program in British Columbia

- Clinician scientists will lead knowledge translation of results to physicians & patients
- Clinician treatment teams will discuss treatment options for individualized therapeutic plans
- Accredited clinical diagnostic laboratory for genetic testing

Pediatric Anthracycline Cardiotoxicity Risk Prediction Tool

- Risk of Cardiotoxicity (%)
  - 89% Cardiotoxicity Risk
  - 45% Cardiotoxicity Risk
  - 39% Cardiotoxicity Risk
  - 21% Cardiotoxicity Risk

Patient Anthracycline-Induced Cardiotoxicity PGx Risk (Percentile)

Canadian Pharmacogenomics Network for Drug Safety

At the Child & Family Research Institute
Children’s & Women’s Health Centre of British Columbia
Vancouver, CANADA
Contact/Questions

Bruce Carleton
Professor and Chair, Div. of Translational Therapeutics
Department of Paediatrics
Faculty of Medicine
University of British Columbia

bcarleton@popi.ubc.ca