Genetic Predictors of Long Term Cardiotoxicity Following Anthracycline Therapy

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Disclosures

• No Conflicts of Interest
ADRs in treatment of childhood cancer:
- 75% of patients suffer one or more ADRs
- 40% of cancer survivors have a severe ADR (life-threatening, or permanently disabling)

Childhood Cancer Survivor Study (2014)

(Armstrong et al. JCO, 2014)
Clinicians treat *individual* patients who vary widely in their drug responses.
ADR Case Report
Case Report:

• A previously healthy 8-year-old child presented with neuroblastoma to B.C. Children’s Hospital

• Began anthracycline (doxorubicin) chemotherapy

• Prior to last cycle of treatment, visited B.C. Children’s Hospital for routine CT scan

• Became unwell during scan
Case Report:

- During CT scan developed serious cardiac dysfunction with virtually no cardiac output
- Intubated and rushed to ICU
- Placed on extracorporeal membrane oxygenation (ECMO) for 3 weeks
- 1 year later received a heart transplant
- First transplanted heart rejected
- Child received a second heart transplant
- Currently in cancer remission
Anthracyclines

- e.g., Doxorubicin, Daunorubicin
- Highly effective cancer therapy
- Significantly increased childhood cancer survival rates
- Administered to 60-70% of childhood cancer patients (leukemias & solid tumors)
- Adults: breast cancer, sarcoma, lymphoma, leukemia, and others
Ewings Sarcoma – High Risk

- 71 pediatric patients
- Median anthracycline cumulative dose of 365 mg/m2
- 21/71 had EF drop to below 50%
- 11/71 had EF drop to below 40%
- 5 cardiac deaths (7% of patients)

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 < 300 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 (BC Cancer 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

![Graph showing probability of cardiotoxicity vs cumulative dose](image-url)
Question: Why does one patient develop cardiotoxicity, while another patient does not?
Classification of Anthracycline-Cardiotoxicity

**Controls**

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

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

**Grade 2 toxicity**: Moderate to severe cardiotoxicity
- 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%

ADR Cases

<table>
<thead>
<tr>
<th>Classification</th>
<th>Anthracycline-Cardiotoxicity</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>No cardiotoxicity, SF ≥30%, ≥5yr follow-up</td>
</tr>
<tr>
<td>Grade 1 toxicity</td>
<td>Shortening fraction 27-30% or Resting ejection fraction 50-60%</td>
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<td>Moderate to severe cardiotoxicity Shortening fraction 15-26% or Resting ejection fraction 40-50%</td>
</tr>
<tr>
<td>Grade 3 toxicity</td>
<td>Symptomatic congestive heart failure Shortening fraction &lt; 15% or Resting ejection fraction &lt; 40%</td>
</tr>
<tr>
<td>Grade 4 toxicity</td>
<td>Congestive heart failure requiring heart transplant or ventricular assist device Resting ejection fraction &lt; 20%</td>
</tr>
</tbody>
</table>
Single Marker Analysis Combined CPNDS Cohort

ADR cases: SF≤26%
Control patients: SF≥30% and 5 yr follow-up time

Covariates: dose, age, gender, RT heart, PC1, PC2

Case  | Control
--- | ---
78    | 266

SLC28A3

Visscher et al, JCO 2011
Two-step analysis approach

**Protective Allele Frequency**
- Controls: 19%
- Cases: 8%

### Discovery
- **Gene**: SLC28A3
- **O.R.**: 0.29
- **P-value**: 0.0071
- n = 156
- P < 0.01

### Replication
- **O.R.**: 0.33
- **P-value**: 0.0072
- n = 188
- P < 0.01

### Combined
- **O.R.**: 0.31
- **P-value**: $1.0 \times 10^{-4}$
- n = 344
- Multiple Testing Corrected P-value = 0.017
Potential mechanism of SLC28A3

Reduced SLC28A3 expression

Less anthracycline into cell

Less ROS and toxic alcohol metabolites

Less toxicity
Can we combine multiple SNPs and clinical variables into a pharmacogenetic risk profile?
## Additional Genetic Variants Associated with Anthracycline Cardiotoxicity

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Combined Cohorts n = 521</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>OR (95% CI)</strong></td>
<td><strong>P-value</strong></td>
</tr>
<tr>
<td>SLC28A3</td>
<td>A/G</td>
<td>0.36 (0.22-0.60)</td>
<td>1.6x10^-5</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>A/C</td>
<td>4.30 (1.97-9.36)</td>
<td>2.4x10^-4</td>
</tr>
<tr>
<td>SULT2B1</td>
<td>A/C</td>
<td>0.56 (0.38-0.81)</td>
<td>0.0015</td>
</tr>
<tr>
<td>SLC28A1</td>
<td>A/G</td>
<td>1.60 (1.18-2.17)</td>
<td>0.0020</td>
</tr>
<tr>
<td>ABCB4</td>
<td>A/G</td>
<td>1.67 (1.15-2.43)</td>
<td>0.007</td>
</tr>
<tr>
<td>ABCC1</td>
<td>A/C</td>
<td>2.40 (1.33-4.33)</td>
<td>0.004</td>
</tr>
<tr>
<td>HNMT</td>
<td>G/A</td>
<td>0.56 (0.37-0.86)</td>
<td>0.005</td>
</tr>
<tr>
<td>SLC10A2</td>
<td>A/G</td>
<td>0.57 (0.38-0.87)</td>
<td>0.006</td>
</tr>
<tr>
<td>HNMT</td>
<td>A/C</td>
<td>1.67 (1.15-2.41)</td>
<td>0.007</td>
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Visscher et al, JCO 2011
Genome-wide study of anthracycline-induced cardiotoxicity: Reveals a highly associated genetic factor in the RARG gene

Stage 1
Odds Ratio: 7.0 (2.9-17.0)
P-value: 4.1x10^{-8}

Aminkeng et al., Nat Gen, 2015
Replicated: Replicated association with a coding variant in nuclear receptor *RARG* (retinoic acid receptor-gamma) associated with anthracycline-induced cardiotoxicity.

**Canada**
- O.R.: 7.0
- P-value: $4 \times 10^{-8}$
- n = 280

**Netherlands**
- O.R.: 4.1
- P-value: 0.0042
- n = 96

**Worldwide**
- O.R.: >9
- P-value: 0.00012
- n = 80

**Combined**
- O.R.: 4.7
- P-value: $4.3 \times 10^{-11}$
- n = 456

Aminkeng et al., *Nat Gen*, 2015
RARG represses Top2b expression

- **TOP2B** implicated in anthracycline cardiotoxicity
  - cardiac Top2b deletion protects mice from ACT
  - cardiac **TOP2B** deletion protects SC-CMs from toxicity

- RARG binds to the Top2b promoter

Zhang S et al, 2012 Nat. Med. 18:1639
Validated: Increased susceptibility to anthracycline-toxicity in hiPSC differentiated heart cells with RARG$^{S427L}$

Increased susceptibility of RARG$^{S427L}$ (rs2229774) carriers to anthracycline-toxicity in human iPSC cardiomyocytes, exhibiting a **5.4-fold lower IC$_{50}$** ($P<0.05$) and a 5-fold lower cell viability at 1 uM doxorubicin ($P<0.01$). (Preliminary results, unpublished).

Heart cells with risk variant die at 5-fold lower dose of doxorubicin
Can we incorporate individual patient genomic information to prevent this ADR?

Implemented a Pilot Pharmacogenomic ADR Prevention Program

Site: B.C. Children’s Hospital

  Anthracycline-induced heart failure
  Cisplatin-induced deafness
Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity

Folefac Aminkeng, Colin J. D. Ross, Shahrad R. Rassekh, Soomi Hwang, Michael J. Rieder, Amit P. Bhavsar, Anne Smith, Shubhayan Sanatani, Karen A. Gelmon, Daniel Bernstein, Michael R. Hayden, Ursula Amstutz, Bruce C. Carleton

CPNDS Clinical Practice Recommendations Group

First published: 30 June 2016  Full publication history
DOI: 10.1111/bcp.13008  View/save citation
Patient Anthracycline-Induced Cardiotoxicity PGx Risk (Percentile)

- **14% Risk** (~23% of population. Risk estimate based upon 139 patients. Includes carriers of protective SLC28A3 variant.)
- **21% Cardiotoxicity Risk** (~60% of population. Risk estimate based upon 356 patients. Includes non-carriers, and carriers of 1 risk variant, or 2 risk + 1 protective variant.)
- **39% Cardiotoxicity Risk** (~13% of population. Risk estimate based upon 80 patients. Includes carriers of 1 risk variant, or 2 risk + 1 protective variant.)
- **45% Cardiotoxicity Risk** (~2% of population. Risk estimate based upon 356 patients. Includes non-carriers, and carriers of 1 risk variant, or 2 risk + 1 protective variant.)
- **89% Cardiotoxicity Risk** (~2% of population. Risk estimate based upon 9 patients. Includes carriers of 1+ RARG and 1+ UGT risk variants.)

*Note: Risk estimates based on patient data and genetic variation analysis.*
Case Report (PGx Era)
Case Report with PGx Testing

• 1 year old presenting with a paraspinal mass behind heart, elevated urinary catecholamines, and MIBG positive mass suggestive of neuroblastoma.

• Biopsy confirmed stage IV myc-amplified neuroblastoma

• Treatment protocol calls for treatment with anthracyclines
Applied predictive pharmacogenetic test before anthracyclines administered:

**PGx-cardiotoxicity risk profile for patient**

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- (~2% of population. Risk estimate based upon 11 patients. Includes carriers of 2 RARG risk variants.)

Patient Anthracycline-Induced Cardiotoxicity PGx Risk (Percentile)
Case Report with PGx Testing

• Pharmacogenomic testing results:
  – Highest genetic risk group for anthracycline toxicity: 89%

• Patient also at high clinical risk for cardiac toxicity
  – Young age
  – Radiation to the tumor would result in high exposure of focal radiation to heart
Case Report with PGx Testing

• Clinicians presented patient at Tumor Board
  – Reviewed risks to patient (both clinical and genomic)
  – Reviewed literature for role of anthracyclines in high risk neuroblastoma in infants

• Decision made to avoid anthracyclines
  – Used a treatment protocol where only 1 dose of anthracycline is part of the study and then omit this dose

• Patient completed therapy and is alive and well, in remission (5 yrs) and with normal cardiac function
Precision Medicine Programme (PMP)

• Approaching every pediatric patient receiving either anthracyclines or cisplatin in BC
• 309 patients enrolled in study (April 2019)

![Count of Patients](image.png)

- 253 Patients enrolled for PGx Testing
- 185 for Anthracyclines
- 32 for Cisplatin
- 36 for Both
Precision Medicine Programme (PMP)

- CPGs published
- PGx risk prediction models developed
- PGx testing implemented
- PGx Results
  - Results returned to oncologists using visual aids (Figure 1 & 2)
- Outcomes
  - Patients followed prospectively to review ADR outcomes and changes to therapy
- Value & Utility
  - Patients/families & oncologists interviewed about the value of their PGx results
Therapy Modifications:

Based on variants in 3 genes: RARG, SLC28A3, UGT1A6
Back to First Case Report (2005): Could we have predicted it?
Pharmacogenetic test for risk of anthracycline-induced cardiotoxicity:

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Ongoing Studies:

• New gene discovery
  – Can we improve on our current 3 gene model

• Expansion to additional centers across Canada
  – Go-PGx Study just launched
  – Genome Canada LSARP funding
  – Rolling out across Canada
  – Role Playing game to look at patient, parent, and provider preferences and trade-offs
>300 patients tested in BCCH
Future Studies:

• Can we replicate this finding in adults?
  – Research Trial at BC Cancer Agency and St Paul’s Hospital
  – Vanderbilt Hospital has replicated RARG finding in adults
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