Pharmacogenomic Prediction of Anthracycline-induced Cardiotoxicity in Childhood Cancer

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Outline

- Background and Rationale

- Aminkeng F et al, Nature Genetics 2015
  Sep;47(9):1079-84

- Importance of SNP & Variation Suite (SVS)
Background and Rationale
The Ideal Medication

Effectively treats or prevents disease

Has no adverse effects
Paradox of Modern Drug Development

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

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

- Safe & Effective + Safe & Effective = Effective
- Safe & Effective + No Response = No Response
- Adverse Drug Reaction
Anthracyclines

- Doxorubicin, Daunorubicin, Idarubicin, Epirubicin, Valrubicin, Mitoxantrone

- Administered to 70% of all childhood cancer patients

- Adjuvant chemotherapy for 50-90% of breast cancer
  - 22,000 patients/year in Canada

- At least 970,000 patients receive it each year (N. America)

Highly effective

- Improved childhood cancer survival: from 30% in 1960s to >80% today
  - 1 in 750 young adults is childhood cancer survivor;

Anthracyclines-induced cardiotoxicity (ACT)

- 1 in 2 patients develop detectable cardiac abnormalities (57%)
- 1 in 5 patients suffer Congestive heart failure
- May require intra-ventricular assist device or heart transplant
- Increased risk in children, especially under 4 years old

Aminkeng F et al, Nature Genetics 2015 Sep;47(9):1079-84
Research Question

What are the genetic factors that modify the risk of ACT and can potentially inform treatment decisions?
Analysis Plan Implemented in SVS from Golden Helix

Step 1: Quality Control (Samples & SNPs) - Exclude poor quality data

Step 2: Discovery - Screening & Prioritisation of Candidates for follow-up

Step 3: Replications – Identification of associated gene region

Step 4: Fine mapping - To identify the causal variants within the region

Step 5: Biological Plausibility – Functional relevance of gene & variant
EXAMPLE FROM ONE OF OUR GWAS

Stage 1. Quality Control (Sample and SNP) using SVS

Before QC: Sample size = 434 patients and 740,000 variants

Detail QC Step takes place right after calling the genotypes

Samples
- Sample Call Rates
- Gender Misspecification Check
- Cryptic Relatedness Verification
- Population Stratification

SNP
- SNP call rates
- Minor allele frequency
- Hardy-weinberg Equilibrium

After QC: Sample size = 280 European ancestry patients and 657,694 variants
GWAS Work Flow Using SVS

Stage 1. Quality Control (Sample and SNP)

Stage 2. Screening and Prioritisation of Candidates for follow-up in an initial patient cohort

**Genetic Association Analyses**
- Genotype distribution
- Regression Analysis
- Multiple testing correction or select top candidates by \( p \)-value

*SVS provides a variety of statistical tests to perform all these analyses which largely depends on your data set and research question*

Screened and Prioritized candidates variants in specific genomic regions for follow-up in an independent patient cohort
GWAS Work Flow Using SVS

Stage 1. Quality Control (Sample and SNP)

Stage 2. Screening and Prioritisation of Candidates for follow-up

3. Identifying associated gene regions using another patient cohort

Genetic Association Analyses
- Genotype distribution
- Regression Analysis
- May or may not implement multiple testing correction

SVS provides a variety of statistical tests to perform all these analyses which largely depends on your data set and research question

We Identified a new genomic region
GWAS Work Flow Using SVS

Stage 1: Quality Control (Samples and SNPs) - Exclude poor quality data

Stage 2: Discovery - Screening and Prioritisation of Candidates for follow-up

Stage 3: Replication - Identifying associated gene regions

Stage 4: Fine mapping to identify the causal variants within the region

Imputation
- Available scripts for import and export of data for any of the imputation software programs
- Data analysis after imputation

Sequencing
- SVS can also analyze sequence data

*The causal or most important variant is studies for the mechanistic basis of the ADR phenotype*
Stage 1
Discovery
Europeans from Canada
N = 280 patients: 32 cases & 248 controls

Stage 2
Replication
Europeans from The Netherlands
N = 96 patients: 22 cases & 74 controls

Stage 3
Replication
- Africans: 11 patients
- Latinos: 23 patients
- A. Canadians: 15 patients
- East Asians: 31 patients

Patients (Children treated with Anthracyclines)
Genotyping: Genome-wide Association Study

- GWAS examines genetic variation across the entire genome
- Unbiased and hypothesis-free
- Specifically target common variations
- Potential to discover novel genes, variants, pathways & inform drug development
- Illumina Infinium HumanOmniExpress assay (738,432 SNPs)
- First genome-wide study of anthracycline-induced cardiotoxicity
Genome-wide Association Study

We screened with statistical tests implemented in SVS and prioritized with $P < 1.0E-05$.
GWAS Uncovered *RARG* as Novel Gene for Cardiotoxicity

**Stage 1 & 2 – Discovery & Replication, European Patients**

Gene | Variant | O.R. | P-value | O.R. | P-value | O.R. | P-value |
--- | --- | --- | --- | --- | --- | --- | --- |
*RARG* | rs2229774 | 7.0 | 4.1E-08 | 4.1 | 0.0042 | 5.4 | 1.2E-09 |

**Stage 3 – Replication, Worldwide:**

**Variant** | **O.R.** | **P-value**
--- | --- | --- |
rs2229774 | > 6 | 0.00012 |

GWAS and imputation identified putative haplotype (5 SNP) associated with ACT.

- rs2229774 (S427L) only coding variant in haplotype

**RARG regulates TOP2B, ACT causative gene**

- We showed that *RARG* transcriptionally regulates *Top2b*

- *Top2b* critical to development of ACT *(Zhang et al. 2012 Nat Med 18:1639)*

- *RARG* variant impaired in *Top2b* regulation

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*Aminkeng F et al, 2015. Nature Genetics Sep;47(9):1079-84*
Summary of Main Findings

- **Genetic Association:** Novel gene (*RARG*), Novel variant (rs2229774) & Novel haplotype (5 SNPs) for ACT

- **Functional Validation:** *RARG* & rs2229774 regulates *Top2b* expression; *Top2b* - known ACT-susceptibility gene

- **Conclusion:** *RARG* rs2229774 is a novel pharmacogenetic biomarker & provides novel insight into the pathophysiology of ACT
Next Steps: Ongoing Projects

- Genetic Association (Drs. Carleton BC, Ross CJD and Aminkeng F): We are currently studying the genetic association of *RARG* rs2229774 in adult breast cancer patients.

- In vitro & in vivo functional studies (Drs. Ross CJD and Bhavsar AP): Mechanistic studies of *RARG* & rs2229774 are ongoing & will inform future drug development in the following ways:
  - Development of less heart failure prone cancer treatments
  - Development of more advanced cardio protectants

- Patients Studies (Dr. Bernstein D): A collaboration with a Stanford-based NIH project is studying the role of *RARG* & rs2229774 in cardiotoxicity using real world patient populations.
Next Steps: Ongoing Projects

- Personalized Medicine Project (Drs. Carleton BC, Rassekh RS, Ross CJD): Pilot project on implementation of PGX Testing
What is currently done?

- Treatment decisions based on clinical risk factors

<table>
<thead>
<tr>
<th>Age at Treatment*</th>
<th>Radiation with Potential Impact to the Heart§</th>
<th>Anthracycline Dose†</th>
<th>Recommended Frequency</th>
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<tbody>
<tr>
<td>&lt;1 year old</td>
<td>Yes</td>
<td>Any</td>
<td>Every year</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>&lt;200 mg/m²</td>
<td>Every 2 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥200 mg/m²</td>
<td>Every year</td>
</tr>
<tr>
<td>1-4 years old</td>
<td>Yes</td>
<td>Any</td>
<td>Every year</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>&lt;100 mg/m²</td>
<td>Every 5 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 to &lt;300 mg/m²</td>
<td>Every 2 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥300 mg/m²</td>
<td>Every year</td>
</tr>
<tr>
<td>≥5 years old</td>
<td>Yes</td>
<td>&lt;300 mg/m²</td>
<td>Every 2 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥300 mg/m²</td>
<td>Every year</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>&lt;200 mg/m²</td>
<td>Every 5 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥200 to &lt;300 mg/m²</td>
<td>Every 2 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥300 mg/m²</td>
<td>Every year</td>
</tr>
<tr>
<td>Any age with decrease in serial function</td>
<td></td>
<td></td>
<td>Every year</td>
</tr>
</tbody>
</table>

Children’s Oncology Group Long-Term Follow-up Guidelines v3.0
Genetic factors improve the prediction of ACT beyond clinical factors and can potentially inform treatment decisions.
Genetic Risk Stratification for Anthracycline-induced Cardiotoxicity in Children

- **14% Cardiotoxicity 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 + 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**
  - (~60% of population. Risk estimate based upon 323 patients. Includes carriers of 1 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.)
Clinical Pharmacogenomic Testing

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

Pharmacogenetic Risk Profile:

- **High risk of ACT**
- **Moderate risk of ACT**
- **Low risk of ACT**

All Patients Treated with Same Drug
Genetic Risk Stratification for Anthracycline-induced Cardiotoxicity in Children

- 89% Cardiotoxicity Risk (~2% of population. Risk estimate based upon 9 patients. Includes carriers of 1+ RARG and 1+ UGT risk variants).
- 45% Cardiotoxicity Risk (~2% of population. Risk estimate based upon 11 patients. Includes non-carriers, and carriers of 1 RARG risk 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).
- 21% Cardiotoxicity Risk (~60% of population. Risk estimate based upon 356 patients. Includes non-carriers, and carriers of protective SLC28A3 variant).
- 14% Risk (~23% of population. Risk estimate based upon 139 patients. Includes carriers of protective SLC28A3 variant).

Patient Anthracycline-Induced Cardiotoxicity PGx Risk (Percentile)
Importance of SNP & Variation Suite
SNP & Variation Suite

- Well suited for big & complex data analysis, visualization & interpretation

- Important application for pharmacogenomic studies to uncover the genetic & mechanistic basis of drug response
10 reasons SVS remains our software of choice

1. Very user friendly and great for beginners
2. Detailed user manuals available which is also easy to use
3. Wide range of statistical tests available
4. Great for data visualization
5. Very fast and have lots of computation power
6. Great variety of data manipulating tools
10 reasons SVS remains our Software of Choice

7. Technical support is great & rapid

8. Lots of tutorials and training available on the website

9. Constantly investing heavily in educations & training of its customers on a wide variety of topics in medical and biomedical sciences

10. With the amount of genetic data growing faster than computation capacity of most standard statistical software packages, SVS is the software of choice, especially for scientist & clinicians who are neither statisticians nor bio-informaticians
ACKNOWLEDGEMENTS
CPNDS Network in Canada

13 Paediatric Sites
- 8 CPNDS
- 5 C17 Sites

13 Adult Sites

VANCOUVER
CFRI/BC Children’s Hospital

EDMONTON
Stollery Children’s Hospital

CALGARY
Alberta Children’s Hospital

WINNIPEG
Winnipeg Children’s Hospital

KINGSTON
Kingston General Hospital

LONDON
Children’s Hospital of Western Ontario

TORONTO
Hospital for Sick Children

HAMILTON
Hamilton Children’s Hospital

ST. JOHN’S
Janeway Children’s Hospital

HALIFAX
IWK Grace Health Centre

MONTREAL
Montreal Children’s Hospital

MONTREAL
Sainte-Justine Hospital

OTTAWA
Children’s Hospital of Eastern Ontario

EDMONTON
Stollery Children’s Hospital

VANCOUVER
CFRI/BC Children’s Hospital

Adults: 3 sites-BCCA, VGH, SPH, KGH, PMH, SUN
5 MS Sites-UBC, WIN, LON, HAL, CHUM

CPNDS Paediatric Surveillance Sites

CPNDS Adult Surveillance Sites

CPNDS Network in Canada 34
International Collaborations

CPNDS Collaborations

CPNDS Planned/Potential Collaborations
Question Time
Backup Slides
## Anthracycline-induced Cardiotoxicity

### Risk Factors

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumulative dose</strong></td>
<td>Cumulative doses $&gt;500$ mg/m$^2$ associated with significantly elevated long term risk</td>
</tr>
<tr>
<td><strong>Length of follow-up</strong></td>
<td>Incidence of clinically significant cardiotoxicity increases progressively post-therapy</td>
</tr>
<tr>
<td><strong>Radiation therapy</strong></td>
<td>Cumulative radiation dose ($&gt;30$ Gy); prior or concomitant anthracycline treatment</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Both young and advanced age at treatment are associated with elevated risk</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Females are at greater risk than males</td>
</tr>
<tr>
<td><strong>Rate of anthracycline administration</strong></td>
<td>Prolonged administration to minimise circulating dose volume may decrease toxicity; results are mixed</td>
</tr>
<tr>
<td><strong>Individual anthracycline dose</strong></td>
<td>Higher individual anthracycline doses are associated with increased late cardiotoxicity, even when cumulative doses are limited</td>
</tr>
<tr>
<td><strong>Type of anthracycline</strong></td>
<td>Liposomal encapsulated preparations may reduce cardiotoxicity. Conflicting data exist about anthracycline analogues and cardiotoxicity differences</td>
</tr>
<tr>
<td><strong>Concomitant therapy</strong></td>
<td>Trastuzumab, cyclophosphamide, bleomycin, vincristine, amsacrine, and mitoxantrone may increase susceptibility/toxicity.</td>
</tr>
<tr>
<td><strong>Pre-existing cardiac risk factors</strong></td>
<td>Hypertension; ischaemic, myocardial, and valvular heart disease; prior cardiotoxic treatment</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td>Diabetes, obesity, renal dysfunction, pulmonary disease, endocrinopathies, electrolyte and metabolic abnormalities, sepsis, infection, pregnancy</td>
</tr>
<tr>
<td><strong>Additional factors</strong></td>
<td>Trisomy 21; African American ancestry</td>
</tr>
</tbody>
</table>

Adapted from: Lipshultz et al. Heart. 2008;94(4):525-33
Paradox of Modern Drug Development

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

2. Physicians treat *individual* patients who can vary widely in their response to drug therapy
50% of newly approved therapeutic health products have **serious ADRs**, discovered only after the product is on the market

- Health Canada, 2007
Reports of Severe ADRs are Increasing

![Graph showing increasing serious adverse drug events and outpatient prescriptions (1998-2005)]

**Figure 1.** Reported serious events vs outpatient prescriptions, 1998-2005.

Moore et al, FDA, 2007