Genetic Components of Predicting Drug Adverse Reactions in the Confederated Salish & Kootenai Tribal Population

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Outline

- How did this partnership arise?
- What is pharmacogenetics?
- Results
- Future directions
Partnership

Montana Cancer Institute
Tribal Health and Human Services
Confederated Salish & Kootenai Tribes
University of Montana
Mayo Clinic-Matt Ames Lab
Adverse Drug Reactions (ADRs)

- An unexpected, unintended response to a drug and which occurs at doses normally used in humans.
- Costs to society-$136 billion a year (FDA)
- In the US, > 2 million hospitalized patients experienced ADRs, more than 100,000 fatalities per year
- Fifth leading cause of death ahead of diabetes, AIDS, pneumonia, accidents and automobile deaths
SIDE EFFECTS INCLUDE DILATED PUPILS, RAPID HEARTBEAT, AND THE LOSS OF ANY SHRED OF DIGNITY...
Pharmacogenetics

- Pharmacogenetics can be defined, as the study of genetic factors that determine if a patient is likely to benefit from, or be adversely affected by, a particular medication.
- The term “personalized medicine” has been used in the popular media to describe this field. A great deal has been learned from studies of polymorphisms in a set of genes that predict the outcome of frontline cancer therapeutics.
- These polymorphisms confer differential responses in patients through altered forms of drug metabolic enzymes, drug transporters, or drug targets.
Polymorphisms

- Changes in the base sequence of DNA that can result in protein changes. (RFLPs, STRs, SNPs)
- Defined as occurring in over 1% of the population.
- Often have no effect.
- Can be used for establishing identity.
- Can be used to predict disease susceptibility.
Example of Pharmacogenetics

- Have you or someone you know used codeine with no apparent effect (pain relief)?
Codeine Metabolism - CYP2D6

• Cytochrome P450 2D6 (CYP2D6) is one enzyme that is required for the metabolism of codeine to morphine
• Most people are “extensive metabolizers” that allows them to effectively metabolism codeine to morphine
Different Types of Metabolizers

• A “Poor Metabolizer” (PM) cannot metabolize codeine (not enough 2D6)
• “Extensive Metabolizers” (EM) metabolize codeine (“most people” / “normal”)
• “Ultrarapid Metabolizer” metabolizes too quickly to be able to effectively benefit from the codeine (too much 2D6)
Populations Missing 2D6

- 5-10% of Caucasians are missing 2D6 enzyme activity making them “poor metabolizers” of codeine. An example of an adverse drug reaction (ineffective therapy).

- What % of CSKT members are missing full CYP2D6 activity? (unknown)
Example A

- Patient is diagnosed with transitional cell carcinoma (kidney tumor).
- Choice of drug is 5-fluoro-uracil.
- Patient is placed on 5-FU therapy without testing for polymorphism.
- Outcome is liver failure and death.
Example B

- Patient is diagnosed with transitional cell carcinoma.
- Choice of drug is 5-fluoro-uracil.
- Patient is tested for polymorphisms in the dihydropyrimidine dehydrogenase and thymidylate synthetase genes.
- Polymorphism predicts high likelihood of liver toxicity.
- Patient is placed on alternative therapy.
- Outcome is favorable.
Gap in Knowledge

- Using pharmacogenomics in order to enhance efficacy and minimize toxicity of cancer drugs is becoming more common.
- However, these options are not readily available to tribal populations because of a lack of data concerning the polymorphism frequencies in this population.
- This study seeks to remedy that lack of data by examining these frequencies in a Tribal population.
- Perhaps more important than the data we hope to gather is the trust that we can build to make it possible for other groups to pursue similar studies.
Example C

• Caucasian patient diagnosed with transitional cell carcinoma.
• Drug chosen is 5-fluoro-uracil
• Data shows that there is a 5% chance Caucasians will possess a variant allele that may cause an adverse drug reaction.
• The testing is expensive and risk is so low that patient and doctor decide it is safe to proceed
Example D

- Salish and Kootenai Tribal (CSKT) member diagnosed with transitional cell carcinoma.
- Drug chosen is 5-fluoro-uracil
- There is no data regarding variant allele frequencies in CSKT members, so doctor assumes risk is similar to other ethnic groups (5%) and proceeds with treatment.
- Patient unexpectedly has liver failure and death because the actual variant allele frequency for CSKT individuals is 12%. (Hypothetical)
- Had variant allele frequency for CSKT populations been known, the doctor could have advised other treatment options, tested for TYMS or DPYD, and patient may have lived.
Goals

• Measure the allele frequencies for polymorphisms in specific genes relevant to predicting clinical outcomes when using routine cancer therapy regimens.

• This will allow us to predict the percentage of Salish-Kootenai patients who will be at risk for adverse reactions with commonly used cancer chemotherapeutic agents.

• If the risk is elevated safeguards can be put in place to reduce risk.
Study Overview

• Collected blood samples from Confederated Salish and Kootenai tribal (CSKT) individuals

• Assessed variant allele frequency for 18 loci used to predict patient outcomes in frontline cancer chemotherapy.

• Several loci exhibited variant allele frequencies significantly different from Caucasian or other ethnic populations

• Confirmed that CSKT population is genetically distinct and has a unique set of pharmacogenomic risks.
Methodology

- Individual and group consent was obtained.
- Blood samples of 5-7 mL were drawn. (Powwows, Health fairs, Job Fairs)
- Blood samples were coded for anonymity.
- Coded samples were transported to the University of Montana laboratories where DNA was isolated from whole blood using the QiaAMP Midi Kit from Qiagen (Valencia, CA).
- An aliquot of purified DNA were shipped to the laboratory of Dr. Matt Ames at Mayo Clinic in Rochester, MN for genotyping.
- Statistical analysis compared CSKT allele distributions to various published populations of other ethnic groups using Fishers exact test.
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<th>Locus</th>
<th>Gene Name</th>
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<th>Drugs Metabolized</th>
<th>Variant</th>
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Results

• The differences in variant allele frequencies for the CSKT population was statistically significant from at least one of the 6 ethnic groups in each of the 18 genes studied.

• The variant allele frequencies for the CSKT population showed more significant differences when compared to African, Asian, and European populations than with African American, Asian American, European American ethnic groups.
The graphs show the variant allele frequencies for different populations:

**TYMS2**
- **CSKT**: The allele frequency is indicated by a purple bar.
- **African American**: The allele frequency is indicated by a red bar with **,**
- **Asian American**: The allele frequency is indicated by a blue bar.
- **European American**: The allele frequency is indicated by a white bar with *****.

**MTHFR 1298**
- **CSKT**: The allele frequency is indicated by a purple bar.
- **African American**: The allele frequency is indicated by a red bar with *****.
- **Asian American**: The allele frequency is indicated by a green bar.
- **European American**: The allele frequency is indicated by a blue bar with **.**
ABC B1 EXON26

Variant Allele Frequency

- CSKT
- African American
- African American
- Asian
- Asian American
- European
- European African

**Significance Levels**

- ***: P < 0.001
- **: P < 0.01
- *: P < 0.05
- No symbol: P > 0.05
Variant Allele Frequency Explanations

- The CSKT populations proved to have a distinct variant allele frequencies from Caucasians and other population groups for many key pharmacogenetically-relevant genes.
- Compared the blood quanta of all samples to the number of variant alleles using the fisher’s exact test (p-value) and found few correlations.
- This may be due to low statistical power, inaccurate self-reported blood quanta, or may be do the fact that the 3 tribes which make up CSKT are themselves distinct from each other.
Results

- Results predict a level of risk of an adverse reaction or improper dosing during the administration of various cancer therapeutics unique to the Salish & Kootenai Tribal population.
- All loci were in Hardy-Weinberg Equilibrium with the exception of ABCB1 exon26, ABCB1 exon21, and CYP2C9*3. This is probably due to linkage.
- MTHFR1298, ABCB1 exon21, TYMS2, NQO1*2, CYP2D6*3, ABCB1 exon12, ABCB1 exon26 had variant allele frequencies that were significantly different than Caucasians indicating a unique risk of adverse drug reactions.

- Why focus on Caucasians (Europeans) in the comparisons?
  - The clinical experience of most oncologists in Montana will be primarily based in this ethnic group.
Conclusion

- Baseline variant allele frequency data for CSKT individuals was established.
- Variant allele frequencies were distinct from other ethnic groups for many key pharmacogenetically-relevant genes.

- The importance of pharmacogenetic testing prior to chemotherapy increases with each allelic difference. (The probability of an individual requiring a different dose or having a different therapeutic outcome can be increased if variant alleles are more common in the population).
Future Directions

- Examine these loci in other tribal or ethnic populations that have not been studied to date.
- Application of these studies outside of cancer (coumadin for cardiac arrhythmias).
- Most future work will be done with NextGen sequencing and will look at complete coding regions and promoter.
- Studies looking at genotype/phenotype correlations need to be done for each drug/enzyme pair to assess the degree to which ethnic background alters the pharmacogenomic prediction simply based on genotype.
- Studies looking at genetic predisposition to cancer in tribal groups.
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