Pharmacogenetics in American Indian Populations

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Northwest–Alaska Pharmacogenomics Research Network
What is Pharmacogenomics?

All patients receive same treatment

Responders
Treat with conventional drug or dose

Non-Responders
Treat with alternative drug or dose

Toxic Responders

Pharmacogenetics in American Indian and Alaska Native Populations

- Prevalence and frequency of pharmacogenetic variation is highly diverse across racial and ethnic populations
  - American Indian and Alaska Native (AI/AN) populations have rarely been included in pharmacogenomics research

- 5.2 million AI/AN people live in the United States
  - AI/AN people experience profound health disparities and often lack access to state of the art health care facilities
  - A full understanding of genetic variation in AI/AN communities is essential if pharmacogenetic testing is to reach its optimal clinical utility in patients from these populations

http://www.census.gov/geo/www/maps/ai2010_wall_map/ai2010_wall_map.html
Challenges of Conducting Genetic Research with Indigenous Communities

- Perception that past health research has provided little benefit to indigenous populations

- Legacy of mistrust of academic research – particularly genetic research
  - Misuse of specimens
  - Implications for identity and shared heritage
  - Invalid, stigmatizing interpretations of genetic contributors to health disparity
  - Unequal control over the research process, data, and samples

Boyer et al., *Clin Pharmacol Ther*, 2011
Finding Common Ground

- Creating joint goals
  - What’s the research question?
  - How should it be studied?
  - What benefits will result?

- Obtaining funding
  - Do partners’ goals align with funders?
  - Is there time for partnership development?
  - Are each partner’s contributions supported?

- Sustaining partnership over time
GOALS: Pharmacogenetics in Rural and Underserved Populations

- To develop collaborative approaches to improve the participation of American Indian and Alaska Native people in pharmacogenetic research
- To evaluate opportunities for pharmacogenetics to benefit rural and underserved populations, which traditionally have been understudied

Community Based Participatory Research  SNP Discovery and Characterization  Clinical and Translational Research

NWA PGRN Northwest–Alaska Pharmacogenomics Research Network
Pharmacogenomics Research with the Confederated Salish and Kootenai Tribes (CSKT)

American Indian or Alaska Native Alone or in Combination as a Percent of County Population: 2010
Partnership between Researchers and CSKT Community

- Establishment of a relationship with CSKT Tribal Health and Human Services
- Sought approval from CSKT Tribal Council before initiation of research
  - Continued “progress” reports and prior authorization of new research directions, grant submissions, and publications
- Initial interest in pharmacogenomics in cancer therapy
Community Based Participatory Research

- CBPR is a key strategy for developing research partnerships with AI/AN communities
Community Based Participatory Research

- Formation of a Community Pharmacogenetics Advisory Council (CPAC)

*Members represent different perspectives across community*

- Build trust and strengthen the partnership between researchers and the CSKT community
- Increase tribal input into the research study
- Give advice about the study approaches to ensure cultural appropriateness
- Discuss tribal interest in pharmacogenetic research and the use of pharmacogenetic testing in health care
Community Based Participatory Research

- Health care provider interviews
  - 17 interviews with Physicians, Nurse Practitioners, Physician Assistants, and Pharmacists
  - Goal: to learn of existing beliefs, attitudes, preferences, and experiences about pharmacogenetics

- Information gathered:
  - Attitudes regarding pharmacogenetic testing (i.e. benefits, risks)
  - What kind of pharmacogenetic tests would be considered valuable? (i.e. drug efficacy, dosage/monitoring, severe adverse drug reactions)
  - How could pharmacogenetic tests be used in the clinical setting? (i.e. barriers, facilitators, and suggestions)
Community Based Participatory Research

- Focus groups with patients receiving care at Tribal Health
  - CSKT members who receive their health care at Tribal Health Clinics (4 focus groups of ~8 people each)
  - Goal: to assess views about pharmacogenetic research and clinical implementation in CSKT people

Examples of Questions:
- What do you think about doctors using pharmacogenetic tests before prescribing a drug (i.e. tamoxifene, warfarin)?
- What do you think about researchers collecting blood from you to look at how genes are involved in reactions to medications?
- What do you think about American Indian people participating in these kinds of studies?
SNP Discovery and Characterization

- **Goal:** Resequencing of CYP2D6, CYP3A4, CYP3A5, and CYP2C9 in a CSKT cohort
- Approval from CSKT Tribal Council, CSKT Tribal Health and Human Services, and UM IRB to recruit volunteers
# Summary of Resequencing

- **CYP2D6** (n=188)
- **CYP3A4, CYP3A5, and CYP2C9** (n=94)

<table>
<thead>
<tr>
<th></th>
<th>CYP2D6</th>
<th>CYP3A4</th>
<th>CYP3A5</th>
<th>CYP2C9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total SNPs identified</strong></td>
<td>67</td>
<td>15</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td><strong>Novel SNPs</strong></td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td><strong>Novel Coding SNPs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- G445R (0.27%)
- K119T (0.57%)
CYP2D6 Pharmacogenomics

- Important in metabolism of many drugs
  - 25% of drugs in clinical are at least partly metabolized
- Highly polymorphic >70 SNPs

- Phenotypes
  - Extensive Metabolizers
  - Poor Metabolizers
  - Intermediate Metabolizers
  - Ultrarapid Metabolizers

**CYP2D6-Dependent Polymorphic Metabolism**

Debrisoquine → 4-hydroxy debrisoquine

![Graph](https://example.com/graph.png)

Adapted from Dahl et al., Pharmacogenetics, 1993
# Genetic Polymorphisms in CYP2D6

<table>
<thead>
<tr>
<th>Variant</th>
<th>Protein Effect</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*1</td>
<td>Wild-type</td>
<td>Normal EM</td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>R296C; S486T</td>
<td>Normal EM</td>
</tr>
<tr>
<td>CYP2D6*2xN</td>
<td>Gene duplication</td>
<td>Increased activity UM</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>Frameshift polymorphism</td>
<td>No activity PM</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>Splicing defect</td>
<td>No activity PM</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>Gene deletion</td>
<td>No activity PM</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>Splicing defect</td>
<td>No activity PM</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>P34S; S486T</td>
<td>Reduced activity IM</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>T107I; R296C; S486T</td>
<td>Reduced activity IM</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>Splicing defect</td>
<td>Reduced activity IM</td>
</tr>
<tr>
<td>CYP2D6*49</td>
<td>P34S; F120I; S486T</td>
<td>Reduced activity IM</td>
</tr>
</tbody>
</table>
## CYP2D6 Resequencing (n=188)

<table>
<thead>
<tr>
<th>Allele</th>
<th>CSKT</th>
<th>European</th>
<th>African American</th>
<th>Japanese</th>
<th>Chinese</th>
<th>Canadian Inuit</th>
<th>Canadian First Nation</th>
<th>Central America (Tepehuano/Mestizos)</th>
<th>South America (Embera/Mapuche)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*1</td>
<td>37.57</td>
<td>28.6 – 83.0</td>
<td>29.7 – 83.0</td>
<td>27.0 – 93.79</td>
<td>17.95 – 38.35</td>
<td>89.10</td>
<td>68.0 – 94.0</td>
<td>38.8 – 99.4</td>
<td>39.9 – 84.9</td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>23.40</td>
<td>15.10 – 40.63</td>
<td>4.20 – 26.90</td>
<td>7.65 – 18.30</td>
<td>8.33 – 16.00</td>
<td>–</td>
<td>–</td>
<td>10.34 – 37.0</td>
<td>18.5 – 23.8</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>0.27</td>
<td>0 – 3.20</td>
<td>0.18 – 0.60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 – 1.44</td>
<td>0</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>20.86</td>
<td>11.3 – 33.40</td>
<td>3.86 – 7.80</td>
<td>0 – 0.77</td>
<td>0 – 1.10</td>
<td>6.7 – 8.3</td>
<td>3.0</td>
<td>0.6 – 19.40</td>
<td>3.6 – 17.8</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>1.34</td>
<td>0 – 6.90</td>
<td>2.80 – 6.90</td>
<td>4.10 – 7.17</td>
<td>2.54 – 9.60</td>
<td>–</td>
<td>–</td>
<td>0.80 – 4.60</td>
<td>0 – 4.20</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>0</td>
<td>0 – 2.10</td>
<td>0 – 0.55</td>
<td>0</td>
<td>0 – 0.50</td>
<td>–</td>
<td>–</td>
<td>0 – 1.20</td>
<td>1.10 – 4.20</td>
</tr>
<tr>
<td>CYP2D6*9</td>
<td>0.80</td>
<td>0 – 3.80</td>
<td>0.18 – 1.15</td>
<td>0</td>
<td>0 – 1.27</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>1.34</td>
<td>0.90 – 8.00</td>
<td>2.70 – 7.50</td>
<td>8.60 – 45.92</td>
<td>22.43 – 64.10</td>
<td>2.30</td>
<td>3.0</td>
<td>0 – 12.45</td>
<td>1.80 – 7.10</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>0</td>
<td>0 – 1.11</td>
<td>13.70 – 26.0</td>
<td>0</td>
<td>0 – 0.21</td>
<td>–</td>
<td>–</td>
<td>0 – 10.20</td>
<td>–</td>
</tr>
<tr>
<td>CYP2D6*28</td>
<td>0.27</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CYP2D6*33</td>
<td>0.53</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CYP2D6*35</td>
<td>1.07</td>
<td>4.80 – 8.50</td>
<td>0.38 – 1.10</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.85</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>11.23</td>
<td>6.90 – 14.00</td>
<td>1.84 – 14.90</td>
<td>0.51 – 2.60</td>
<td>2.20 – 4.00</td>
<td>–</td>
<td>–</td>
<td>2.54</td>
<td>–</td>
</tr>
<tr>
<td>CYP2D6*49</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>0.35</td>
<td>0 – 0.60</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

– denotes not determined
# CYP2D6 Genotyping (n=188)

- 37 distinct genotypes identified
- Assigned an activity score to each to predict phenotype

<table>
<thead>
<tr>
<th>Activity Score</th>
<th>Examples</th>
<th>Phenotype Prediction</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>*1/*2 (x2), *2/*33 (x2)</td>
<td>UM</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>*1/*1, *1/*2, *2/*2</td>
<td>EM</td>
<td>36.36</td>
</tr>
<tr>
<td>1.5</td>
<td>*1/*41, *2/*41, *1/*10, *2/*10</td>
<td>EM</td>
<td>19.24</td>
</tr>
<tr>
<td>1 or 2</td>
<td>*1/*4 (x2)</td>
<td>EM</td>
<td>1.07</td>
</tr>
<tr>
<td>0.5</td>
<td>*4/*41, *5/*41</td>
<td>IM</td>
<td>3.20</td>
</tr>
<tr>
<td>0</td>
<td>*4/*4</td>
<td>PM</td>
<td>5.88</td>
</tr>
<tr>
<td>ND</td>
<td>coding variants of unknown function</td>
<td>ND</td>
<td>2.65</td>
</tr>
</tbody>
</table>
CYP3A Pharmacogenomics

- >50% of all drugs that undergo CYP metabolism are metabolized by CYP3A

1. CYP3A4
   - Most prominent
   - Huge interindividual variability

2. CYP3A5
   - High degree of genetic variability among ethnic groups

CYP3A4 and CYP3A5 Resequencing (n=94)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Protein Effect</th>
<th>CSKT</th>
<th>CEU</th>
<th>YRI</th>
<th>JPT</th>
<th>CHB</th>
<th>Central America (Tepehuano/Mestizos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4*1B</td>
<td>5'-UTR</td>
<td>2.20</td>
<td>3.0</td>
<td>72.0</td>
<td>0</td>
<td>0.3</td>
<td>8.0 – 8.8</td>
</tr>
<tr>
<td>CYP3A4*15A</td>
<td>R162Q</td>
<td>0.68</td>
<td>0</td>
<td>2.84</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>CYP3A4*22</td>
<td>non-coding</td>
<td>2.44</td>
<td>5.29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>CYP3A4*1G</td>
<td>non-coding</td>
<td>26.81</td>
<td>8.3</td>
<td>88.9</td>
<td>29.7</td>
<td>28.0</td>
<td>–</td>
</tr>
<tr>
<td>CYP3A4*13</td>
<td>P416L</td>
<td>0.60</td>
<td>0.4</td>
<td>0</td>
<td>0.6</td>
<td>1.2</td>
<td>–</td>
</tr>
<tr>
<td>CYP3A5*1</td>
<td>wild-type</td>
<td>7.53</td>
<td>5.8</td>
<td>84.8</td>
<td>26.3</td>
<td>33.5</td>
<td>–</td>
</tr>
<tr>
<td>CYP3A5*6</td>
<td>slicing defect</td>
<td>0</td>
<td>0</td>
<td>16.8</td>
<td>0.6</td>
<td>1.2</td>
<td>–</td>
</tr>
<tr>
<td>CYP3A5*7</td>
<td>slicing defect</td>
<td>0</td>
<td>0</td>
<td>0†</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

– denotes not determined

Allele frequencies from HapMap Project. CEU: Europe; YRI: Yoruba; JPT: Japanese; CHB Han Chinese
## CYP3A Linkage Disequilibrium

<table>
<thead>
<tr>
<th>SNP Pairs</th>
<th>CSKT</th>
<th>CEU</th>
<th>YRI</th>
<th>JPT</th>
<th>CHB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP3A4<em>1G vs. CYP3A5</em>1</strong></td>
<td>0.158</td>
<td>0.681</td>
<td>0.387</td>
<td>0.654</td>
<td>0.591</td>
</tr>
<tr>
<td><strong>CYP3A4<em>1B vs. CYP3A5</em>1</strong></td>
<td>0.052</td>
<td>0.414</td>
<td>0.100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>CYP3A4<em>1G vs. CYP3A4</em>1B</strong></td>
<td>0.004</td>
<td>0.282</td>
<td>0.207</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

– Denotes not determined because *CYP3A4*1B not present in JPT and very low in CHB.
**CYP2C9 Resequencing (n=94)**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Protein Effect</th>
<th>CSKT</th>
<th>CEU</th>
<th>YRI</th>
<th>JPT</th>
<th>CHB</th>
<th>Canadian Inuit</th>
<th>Canadian First Nation</th>
<th>Central America (Tepehuano/Mestizos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>R144C</td>
<td>5.17</td>
<td>10.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.0</td>
<td>1 – 7</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>I359L</td>
<td>2.69</td>
<td>5.8</td>
<td>0</td>
<td>2.3</td>
<td>4.7</td>
<td>0</td>
<td>6.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Allele frequencies from HapMap Project. CEU: Europe; YRI: Yoruba; JPT: Japanese; CHB Han Chinese
Summary of CSKT Pharmacogenetic Variation

- Population frequencies may help explain differences in drug metabolism in the CSKT population
- Previously unidentified variants found only in low frequencies in CYP genes
- Allele frequencies were found to be different between the CSKT and other studied populations
  - Frequencies of common alleles in the \textit{CYP2D6}, \textit{CYP2C9} and \textit{CYP3A5} genes were similar to those observed in European Americans
  - Marked divergence in allelic frequencies between CSKT and European populations at the \textit{CYP3A4*1G} locus
- Allele frequencies in one population cannot be used as a surrogate for another
Clinical and Translational Research

- Genotype-phenotype associations in CSKT participants
  1. Tamoxifen pharmacogenomics
  2. *CYP3A4*/*I*G pharmacokinetic study
Tamoxifen Pharmacogenomics

- Prodrug that must undergo metabolic activation to elicit pharmacological activity
- Endoxifen is active metabolite
- **CYP2D6** is the primary enzyme responsible for the activation
- **CYP3A4**, **CYP3A5**, and **CYP2C9** also involved
Tamoxifen Pharmacogenomics

- Genotype-phenotype associations in CSKT patients with breast cancer taking tamoxifen

Pharmacokinetics Study
- Genotype CYP2D6, CYP3A4, CYP3A5, and CYP2C9
- Measure steady-state circulating levels of tamoxifen, endoxifen, and other metabolites
  - (E)- and (Z)-isomers of hydroxylated metabolites
- Are endoxifen levels associated with the genotypes of major cytochrome P450s?
CYP3A4*1G Pharmacokinetic Study

- Genotype-phenotype associations of CYP3A4*1G in health volunteers
  - Reduced function CYP3A4*1G (26.81%)
  - Non-functional CYP3A5*3 (92.47%)

- Pharmacokinetic Study
  - Genotype CYP3A4 and CYP3A5
  - Single 2 mg oral midazolam dose
  - Measure plasma and urine concentrations of midazolam and 1-OH-midazolam
  - Does CYP3A4*1G alter clearance?
Conclusions

- Rationale for pharmacogenetic studies in indigenous people is compelling
  - Novel gene variation and allele frequencies can emerge
  - Influence the focus and structure of the use pharmacogenetic tests to improve health outcomes

- Application of the CBPR model is an effective and respectful way to conduct pharmacogenetic research with indigenous people
  - Develops long-term, trusting partnerships
  - Builds research capacity in communities
  - Helps to identify the priorities of the communities to address health disparities
Acknowledgements

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