Pharmacogenomics-based individualization of drug therapy

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Director

- Research Group for Genotyping
- Research Group for Medical Informatics
- Research Group for Disease-Causing Mechanism
- Research Group for Pharmacogenomics

Our mission is to understand individual gene variation and to apply this knowledge to healthcare in order to promote full and healthy lives.
Response rates of patients to a major drug:
Physician's Desk Reference (2000)

Spear et al. TRENDS in Molecular Medicine 7: 201-4 (2001)
Individualization of drug therapy in personalized medicine

Drug A:

- Effective
- Not effective

To identify:
- Responders / non-responders to a drug
- Patients with higher risk of adverse drug reactions (ADRs) before the administration

“Right drug at the appropriate dosage for each individual patient”
Pharmacogenomics (PGx): ICH Guideline E15

- Study of **variations of DNA and RNA characteristics** as related to drug response

- Applicable to activities such as drug discovery, drug development, and clinical practice
Application of PGx to personalized medicine

1. Avoid severe adverse drug reactions
   ✓ Docetaxel (anti-cancer reagent)

2. Predict efficacy
   ✓ Tamoxifen (treatment for breast cancer)

3. Predict appropriate dosage for each patient
   ✓ Warfarin (anti-coagulant)
Docetacel (Taxotere®)

- Microtubule inhibitor
- Approved for treatment of patients with refractory breast cancer, non-small-cell lung cancer, ovarian cancer, and head and neck cancer
- Severe myelosuppression (leucopenia / neutropenia): Frequency of ~36%
Transporters responsible for biliary excretion of docetaxel

Systemic circulation

Liver

Hepatic artery

Bile duct

Excretion

Hepatic vein

Blood

Hepatocyte

SLCO1B3

Docetaxel

ABCC2

Hepatocyte

Bile duct
SNPs in *ABCC2* and *SLCO1B3* were associated with docetaxel-induced myelosupression

<table>
<thead>
<tr>
<th></th>
<th>ADR (n = 39)</th>
<th>Non-ADR (n = 74)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABCC2</strong> (rs12762549)</td>
<td>GG + GC</td>
<td>CC</td>
<td>GG + GC</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>(62%)</td>
<td>(38%)</td>
<td>(89%)</td>
</tr>
<tr>
<td><strong>SLCO1B3</strong> (rs11045585)</td>
<td>AA</td>
<td>AG + GG</td>
<td>AA</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>(51%)</td>
<td>(49%)</td>
<td>(85%)</td>
</tr>
</tbody>
</table>
Prediction system for docetaxel-induced myelosupression using ABCC2 rs12762549 and SLCO1B3 rs11045585

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Genotype</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11 12 22</td>
<td></td>
</tr>
<tr>
<td>SLCO1B3</td>
<td>rs11045585</td>
<td>0 1 1</td>
<td></td>
</tr>
<tr>
<td>ABCC2</td>
<td>rs12762549</td>
<td>0 0 1</td>
<td></td>
</tr>
</tbody>
</table>

OR = 7.00, P = 0.0000057

ADR (n = 39)
Non-ADR (n = 74)
Normal control (n = 932)

Contraindication
Decreasing dosage
Usual dosage
Tamoxifen (Nolvadex®)

- Widely used for prevention of recurrence for patients with estrogen receptor-positive breast cancer (adjuvant hormonal therapy)

- Potent in suppressing estrogen-dependent cell proliferation

- Oral administration, 20～40 mg/day
Tamoxifen, antagonist for estrogen receptor

Premenopausal

Hypophysis

Postmenopausal

Stimulating hormone

Ovary

Androgen

Adrenal

Estrogen

Aromatase

Breast cancer

Tamoxifen

Estrogen
Metabolic activation of tamoxifen by CYP2D6

### High frequency of CYP2D6*10 in Japanese

<table>
<thead>
<tr>
<th>Allele</th>
<th>Allele freq. in Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*1</td>
<td>Normal</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>A2549del</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>G1846A (splicing defect)</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Whole deletion</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>Decreased 40.8</td>
</tr>
<tr>
<td></td>
<td>C100T (P34S)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Allele freq. in Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total 6.6 (homozygote, &lt;1%)</td>
</tr>
</tbody>
</table>

- Normal
- None
- A2549del
- G1846A (splicing defect)
- Whole deletion
- Decreased 40.8
- C100T (P34S)
### CYP2D6 genotype frequencies in Japanese breast cancer patients treated with tamoxifen

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of Subjects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1</td>
<td>20 (29.9)</td>
</tr>
<tr>
<td>*1/*4</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>*1/*5</td>
<td>4 (6.0)</td>
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<tr>
<td>*1/*10</td>
<td>23 (34.3)</td>
</tr>
<tr>
<td>*5/*10</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>*5/*41</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>*10/*10</td>
<td>15 (22.4)</td>
</tr>
<tr>
<td>*10/*21</td>
<td>1 (1.5)</td>
</tr>
</tbody>
</table>
Effects of CYP2D6*10 on recurrence-free survival of breast cancer patients treated with tamoxifen

\[ P = 0.0031 \]

\[ P = 0.0010 \]
Warfarin (WF)

- The most commonly-used oral anticoagulant for treatment of thromboembolism in the world

- Interferes with regeneration of vitamin K → inhibits activation of vitamin K-dependent clotting factor II (prothrombin), VII, IX and X

- Difficult to adjust the appropriate dose to each patient due to large interindividual variation in dose requirement
  Insufficient dose → failure of preventing thrombosis
  Over-dose → increase of unexpected bleeding risk

- The maintenance dose for Japanese was about 30% lower than those for Caucasian
Impact of inappropriate dose of WF in USA

2 million people / year start warfarin treatment

Bleeding event:
184,000 people (9%)

Cost: $2.4 billion

Stroke:
40,000 (2%)

Cost: $1.6 billion

Cost due to inappropriate dosage:
$4.0 billion / year

McWilliam et al.
Distribution of daily maintenance dose in WF-treated 828 Japanese patients

Median: 2.5 mg
Minimum: 0.5 mg
Maximum: 10.5 mg
Flows of standard WF treatment

INR monitoring

e.g., Prevention of stroke caused by atrial fibrillation in elder patients: 1.6 - 2.6

Initial dosage: 1 mg

A few days after

INR: Within target range?

A few days after

Yes

Continue the dosage

No

Increase / decrease / discontinue
Warfarin PGx: CYP2C9 and VKORC1

- **CYP2C9**
  - Hepatic drug-metabolizing enzyme of warfarin

- **VKORC1**
  - Responsible for recycle of vitamin K in liver
The vitamin K cycle

Reduced form

Vitamin K epoxide

Factors II, VII, IX, X

Proteins C, S, Z

Functional factors

Inhibition

VKORC1
Daily WF dose for Japanese patients with different genotypes for CYP2C9

\[ P = 3.9 \times 10^{-4} \]
Daily WF dose for Japanese patients with different genotypes for VKORC1

Intron 1-136T>C

- CC (n=6)
- CT (n=132)
- TT (n=690)

Dose (mg/day)

Japanese: <1% 16% 84%
Caucasian: 37% 48% 15%

$P = 5.1 \times 10^{-11}$

(Wadelius et al., Pharmacogenomics J, 2005)
Daily WF dose for Japanese patients classified by Warfarin responsive index (WFRI)

<table>
<thead>
<tr>
<th>VKORC1</th>
<th>TT</th>
<th>CT</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CYP2C9</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>*3/*3</td>
<td>*3/*1</td>
<td>*1/*1</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Score 0  Score 1  Score 2

P = $4.4 \times 10^{-13}$

Dose (mg/day)

<table>
<thead>
<tr>
<th>Score 0 (n=35)</th>
<th>Score 1 (n=658)</th>
<th>Score 2 (n=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 mg
Prediction of initial WF dose based on WFRI

Genotyping
VKORC1 and CYP2C9

Score 0
Started with 2 mg

Score 1
With 2.5 mg

Score 2
With 3.5 mg

Although the dose adjustment with INR is necessary, ....

- Reducing period for achievement of maintenance dose
- Avoiding risk of serious bleeding / stroke
Invader assay
Prediction of appropriate dosage by genotyping based on Invader assay

Genotype

SNP1
FAM
VIC
SNP2
SNP3

Fluorescence
Time

SNP analysis system

Genotype
CC
CT
TT

Transfer

Patient ID: 12345
Initial dosage: X mg

Medical treatment support system
FOR IMMEDIATE RELEASE
August 16, 2007

FDA Approves Updated Warfarin (Coumadin) Prescribing Information

New Genetic Information May Help Providers Improve Initial Dosing Estimates of the Anticoagulant for Individual Patients

The U.S. Food and Drug Administration announced today the approval of updated labeling for the widely used blood-thinning drug, Coumadin, to explain that people’s genetic makeup may influence how they respond to the drug.

Manufacturers of warfarin, the generic version of Coumadin, are to add similar information to their products’ labeling, FDA said.

The labeling change highlights the opportunity for healthcare providers to use genetic tests to improve their initial estimate of what is a reasonable warfarin dose for individual patients. Testing may help optimize the use of warfarin and lower the risk of bleeding complications from the drug.

These labeling updates are based on an analysis of recent studies that found people respond to the drug differently based, in part, on whether they have variations of certain genes.

FDA estimates that 2 million persons start taking warfarin in the United States every year to prevent blood clots, heart attacks and stroke. Warfarin is a difficult drug to use because the optimal dose varies and depends on many risk factors including a patient’s diet, age, and the use of other medications.

Patients who take a dose larger than they can tolerate are at risk of life-threatening bleeding. Those who receive too low a dose are at risk of equally dangerous blood clots. Dosing is particularly important at the beginning of therapy, when problems in adjusting the dose can lead to complications such as bleeding.

Warfarin is the second most common drug—after insulin—implicated in emergency room visits for adverse drug events.

Physicians and other health care professionals who prescribe warfarin regularly check to see if the drug is working properly by ordering a test called the PT or prothrombin time that evaluates the blood’s ability to clot properly. The results are measured in seconds and compared with the expected value in healthy people, known as the International Normalized Ratio or INR.

“Today’s approved labeling change is one step in our commitment to personalized medicine. By using modern science to get the right drug in the right dose for the right patient, FDA will further enhance the safety and effectiveness of the medicines Americans depend on,” said Commissioner of Food and Drugs Andrew C. van Eschbach, M.D.

The FDA’s “personalized medicine” initiative makes use of pharmacogenomics—the science that predicts a response to drugs based upon a person’s genetic makeup. This effort supports the personalized health program spearheaded by Health and Human Services Secretary Mike Leavitt.

A person’s genes “encode” enzymes and differences in the sequence of a gene can cause differences in enzyme activity or sensitivity. That is why different people process the
Considerations for Increased Bleeding Risk

Identification of risk factors for bleeding and certain genetic variations in \textit{CYP2C9} and \textit{VKORC1} in a patient may increase the need for more frequent INR monitoring and the use of lower warfarin doses.

Initial Dosage

The lower initiation doses should be considered for patients with certain genetic variations in \textit{CYP2C9} and \textit{VKORC1} enzymes as well as for elderly and/or debilitated patients and patients with potential to exhibit greater than expected PT/INR responses to COUMADIN.