Phenotyping for Pharmacogenomics and Precision Medicine

Nigel Clarke PhD
Learning Objectives

1. To understand the concept of the personal phenotype and how it can be utilized

2. To understand how the personal phenotype impacts the metabolism/distribution/effect of a drug in a person

3. To know how to and when to use pharmacogenetics, personal phenotype determination or a combination of both.
Disclaimer

• Employee of Quest Diagnostics
• Own stock in Quest Diagnostics

• Do own my soul…
What is a personal “Phenotype”

- The Genetic Code of a person can be thought of as the blueprint
- The proteins generated from the DNA can be thought of the house built from the blueprint
- However, we are what is referred to in the US Real Estate business as “semi-custom”…
- We all have roughly the same build BUT we have had custom “features” added…
- …Which can be thought of as your own PERSONAL phenotype
Genotype vs Phenotype…

What you planned (On Budget)

After your spouse’s “tweaks” (And you are Bankrupt!)
Here are other examples...

Genotype (What could be)
Phenotype (The Sad Reality)
The REALLY Sad Reality!
So why does it matter?

- There are many known mutations in the CYP system for example
- Some of those lead to up-regulated activity, most lead to down-regulated activity
- Since they are known they can be tested for during drug development and accounted for.
- That is the genotype approach.
CYP 2D6 background

- The gene is located in chromosome 22
- Four Distinct Phenotypes: UM, EM, IM and PM
- 5-10% of Caucasians are PMs, and 10-15% are IMs
- Approximately 12 alleles confer poor metabolizer phenotype
- PM have lower production of Endoxifen in general
- UM have high levels of endoxifen and high norendoxifen (potent AI)
CYP 2D6 Mutations and their effect and prevalence

<table>
<thead>
<tr>
<th>Allele</th>
<th>Enzyme Activity</th>
<th>Caucasian</th>
<th>African American</th>
<th>Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>*4</td>
<td>None</td>
<td>18-23%</td>
<td>7-9%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>*5</td>
<td>None</td>
<td>2-4%</td>
<td>6-7%</td>
<td>5-6%</td>
</tr>
<tr>
<td>*6</td>
<td>None</td>
<td>1%</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>*10</td>
<td>Reduced</td>
<td>4-8%</td>
<td>3-8%</td>
<td>39-41%</td>
</tr>
<tr>
<td>*17</td>
<td>Reduced</td>
<td>N/A</td>
<td>15-26%</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Phenotype vs Genotype

- However...

- There are also many external factors that impact the activity of the CYP enzymes – both the native and mutant forms

- That will NOT be picked up by the genotype and is fluid depending on the person’s habits/lifestyle/co-morbidities/co-medications
Known 2D6 inhibitors/activity modifiers

### Physician Guidelines: Drugs Metabolized by Cytochrome P450’s

#### 2D6 Substrates

<table>
<thead>
<tr>
<th>Acetaminophen</th>
<th>Captopril</th>
<th>Dextroamphetamine</th>
<th>Fluphenazine</th>
<th>Methoxyphenamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajmaline</td>
<td>Carteol</td>
<td>Dextromethorphan</td>
<td>Fluvoxamine</td>
<td>Metoclopramide</td>
</tr>
<tr>
<td>Alpenrolol</td>
<td>Carvediol</td>
<td>Diazinon</td>
<td>Galantamine</td>
<td>Paroxetine</td>
</tr>
<tr>
<td>Amenifiline</td>
<td>Cevimeline</td>
<td>Dihydrcodeine</td>
<td>Guanoxan</td>
<td>Perhexiline</td>
</tr>
<tr>
<td>Amtriptyline</td>
<td>Chlorpromazine</td>
<td>Diltiazem</td>
<td>Haloperidol</td>
<td>Perphenazine</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Chlorpheniramine</td>
<td>Diprafenone</td>
<td>Hydrocodone</td>
<td>Phenacetin</td>
</tr>
<tr>
<td>Anpranolavir</td>
<td>Chlorpromylide</td>
<td>Dolasetron</td>
<td>Ibovaine</td>
<td>Phenformin</td>
</tr>
<tr>
<td>Aprindine</td>
<td>Cinnarizine</td>
<td>Donepezil</td>
<td>Iloperidone</td>
<td>Timolol</td>
</tr>
<tr>
<td>Arapiproxate</td>
<td>Citalopram</td>
<td>Doxepin</td>
<td>Imipramine</td>
<td>Tolerodine</td>
</tr>
<tr>
<td>Atomoxetine</td>
<td>Clomipramine</td>
<td>Encainide</td>
<td>Indoramin</td>
<td>Tolterodine</td>
</tr>
<tr>
<td>Benztropine</td>
<td>Clozapine</td>
<td>Ethylmorphine</td>
<td>Lidocaine</td>
<td>Trandolol</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>Codeine</td>
<td>Ezlopitant</td>
<td>Loratadine</td>
<td>Tramadol</td>
</tr>
<tr>
<td>Brotarane</td>
<td>Debrisoquine</td>
<td>Flecainide</td>
<td>Maprotiline</td>
<td>Trimipramine</td>
</tr>
<tr>
<td>Bufuralol</td>
<td>Delavirdine</td>
<td>Flunarizine</td>
<td>Meclazine</td>
<td>Triclofosnaide</td>
</tr>
<tr>
<td>Busetrolol</td>
<td>Desipramine</td>
<td>Fluoxetine</td>
<td>Methadone</td>
<td>Terazosine</td>
</tr>
<tr>
<td>Butylamphetamine</td>
<td>Dextenfuranine</td>
<td>Flupenelpan</td>
<td>Methamphetamine</td>
<td>Terbinafine</td>
</tr>
</tbody>
</table>

#### 2D6 Inhibitors

<table>
<thead>
<tr>
<th>Ajmaline</th>
<th>Chlorpromazine</th>
<th>Diphenhydramine</th>
<th>Indinavir</th>
<th>Mibefradil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>Cimetidine</td>
<td>Doxorubicin</td>
<td>Lasoprazole</td>
<td>Modinodine</td>
</tr>
<tr>
<td>Amtriptyline</td>
<td>Citrapride</td>
<td>Fluoxetine</td>
<td>Levomepromazine</td>
<td>Nelfinavir</td>
</tr>
<tr>
<td>Aprindine</td>
<td>Citrapram</td>
<td>Fluphenazine</td>
<td>Lopinavir</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>Azelastine</td>
<td>Clozapine</td>
<td>Fluvastatin</td>
<td>Lortatide</td>
<td>Nicardipine</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Clozapine</td>
<td>Fluvoxamine</td>
<td>Mequazoline</td>
<td>Norflxoxelte</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>Cocaine</td>
<td>Haloperidol</td>
<td>Methadone</td>
<td>Paroxetine</td>
</tr>
<tr>
<td>Chlorpromiramine</td>
<td>Desipramine</td>
<td>Imipramine</td>
<td>Metoclopramide</td>
<td>Pimozide</td>
</tr>
</tbody>
</table>

#### 2D6 Inducers

- Dexamethasone
- Rifampin

**Substrates refer to drugs that are either activated or deactivated by the pathway. Inhibitors refer to drugs that reduce the ability of the pathway to process drugs. Co-administration will decrease the rate of metabolism of drugs through the metabolic pathway listed, increasing the possibility of toxicity. Inducers refer to drugs that increase the activity of a pathway. Co-administration increases the rate of excretion of drugs metabolized through the pathway indicated, reducing the drugs effectiveness. Note: bold = major; italics = minor**
# Known 2C9 inhibitors/activity modifiers

## 2C9 Substrates
- Aceclofenac
- Acenocoumarol
- Alosetron
- Amisulpride
- Amotriptyline
- Amprenavir
- Antipyrine
- Candesartan
- Carmusine
- Carvedilol
- Celecoxib
- Cyclophosphamide
- Dapsone
- Desogestrel
- Dextrimethorphan
- Diclofenac
- Diltiazem
- Flubiprofen
- Fluoxetine
- Fluvasstatin
- Glimepiride
- Glipizide
- Gliburide
- Halofantrine
- Ibuprofen
- Indomethacin
- Irbecartan
- Ketoprofen
- Losartan
- Metenamic acid
- Meloxicam
- Mephobarbital
- Mesranol
- Methadone
- Naproxen
- Paclitaxel
- Phenacetin
- Phenyltoin
- Piroxicam
- Progestosterone
- Rosiglitazone
- Sertraline
- S-naproxen
- Suprofen
- S-Warfarin
- Tamoxifen
- Tetrahydrocannabinol
- Tobutamide
- Torsemide
- Tramipramine
- Valdecoxib
- Valproic acid
- Valsartan
- Warfarin
- Zafirlukast
- Zileuton
- Zolpidem

## 2C9 Inhibitors
- Amiodarone
- Anastrazole
- Cimelidine
- Clopidogrel
- Delavirdine
- Efavirenz
- Fluconazole
- Fluoxetine
- Fluvasstatin
- Fluvoxamine
- Isoniazid
- Lasoprazone
- Loratidine
- Lovastatin
- Modafinil
- Nicardipine
- Nifedipine
- Paroxetine
- Phenylbutazone
- Probenidic
- Ranilidene
- Ritonavir
- Rodilidene
- Sulfaphenazole
- Sulfispyrazone
- Teniposide
- Trimethoprim
- Valproic acid
- Zafirlukast

## 2C9 Inducers
- Carbamezepine
- Cyclophosphamide
- Dexamethasone
- Ethanol
- Ifosfamide
- Phenobarbital
- Phenyltoin
- Rifabutin
- Rifampin
- Ritonavir

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Substrates refer to drugs that are either activated or deactivated by the pathway. Inhibitors refer to drugs that reduce the ability of the pathway to process drugs. Co-administration will decrease the rate of metabolism of drugs through the metabolic pathway listed, increasing the possibility of toxicity. Inducers refer to drugs that increase the activity of a pathway. Co-administration increases the rate of excretion of drugs metabolized through the pathway indicated, reducing the drugs' effectiveness.

Note: bold = major; italics = minor
### Known 3A4 inhibitors/activity modifiers

#### Examples of CYP3A Inhibitors

<table>
<thead>
<tr>
<th>Generic</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antidepressants</strong></td>
<td></td>
</tr>
<tr>
<td>nefazodone</td>
<td>Serzone®</td>
</tr>
<tr>
<td><strong>Antifungals</strong></td>
<td></td>
</tr>
<tr>
<td>itraconazole</td>
<td>Sporanox®</td>
</tr>
<tr>
<td>ketoconazole</td>
<td>Nizoral®</td>
</tr>
<tr>
<td>voriconazole</td>
<td>Vfend®</td>
</tr>
<tr>
<td><strong>Antivirals</strong></td>
<td></td>
</tr>
<tr>
<td>atazanavir</td>
<td>Reyataz®</td>
</tr>
<tr>
<td>indinavir</td>
<td>Crizalve®</td>
</tr>
<tr>
<td>nelfinavir</td>
<td>Viracept®</td>
</tr>
<tr>
<td>ritonavir</td>
<td>Norvir®</td>
</tr>
<tr>
<td>saquinavir</td>
<td>Invirase®</td>
</tr>
<tr>
<td><strong>Macrolide Antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>clarithromycin</td>
<td>Biaxin®</td>
</tr>
<tr>
<td>telithromycin</td>
<td>Ketek®</td>
</tr>
<tr>
<td><strong>Other Agents</strong></td>
<td></td>
</tr>
<tr>
<td>grapefruit juice</td>
<td>N/A</td>
</tr>
</tbody>
</table>

#### Examples of CYP3A Inducers

<table>
<thead>
<tr>
<th>Generic</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anticonvulsants</strong></td>
<td></td>
</tr>
<tr>
<td>carbamazepine</td>
<td>Tegretol®</td>
</tr>
<tr>
<td>phenobarbital</td>
<td>N/A</td>
</tr>
<tr>
<td>phenytoin</td>
<td>Dilantin®</td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>rifampin/rifampicin</td>
<td>Rifadin®</td>
</tr>
<tr>
<td>rifabutin</td>
<td>Mycobutin®</td>
</tr>
<tr>
<td><strong>Other Agents</strong></td>
<td></td>
</tr>
<tr>
<td>St. John’s Wort (Hypericum perforatum)</td>
<td>N/A</td>
</tr>
<tr>
<td>dexamethasone</td>
<td>Decadron®</td>
</tr>
</tbody>
</table>
So what has this got to do with Tamoxifen?
Tamoxifen and Breast Cancer
Breast Cancer - Background

- About 1 in 8 U.S. women (about 12%) will develop invasive breast cancer over the course of her lifetime.

- In 2017, an estimated 252,710 new cases of invasive breast cancer are expected to be diagnosed in women in the U.S., along with 63,410 new cases of non-invasive (in situ) breast cancer.

- About 40,610 women in the U.S. are expected to die in 2017 from breast cancer, though death rates have been decreasing since 1989. Women under 50 have experienced larger decreases. These decreases are thought to be the result of treatment advances, earlier detection through screening, and increased awareness.

- For women in the U.S., breast cancer death rates are higher than those for any other cancer, besides lung cancer.

- Besides skin cancer, breast cancer is the most commonly diagnosed cancer among American women. In 2017, it's estimated that about 30% of newly diagnosed cancers in women will be breast cancers.
Tamoxifen History

- First developed in 1950 as a potential morning after pill due to anti-estrogen action
- Eventually got approved as a fertility treatment!
- Walpole (head of group @ICI) kept it alive despite lack of patent protection
- First clinical study in 1971 showed good effect in breast cancer – but languished
- Finally in 1980 first trial to show anti-cancer effect when used in addition to chemo published
- Later in 1980s SERM action described and accepted – Tamoxifen was reborn.
Tamoxifen as a drug

- It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system.

- Patients with variant forms of the gene CYP2D6 may not receive full benefit from tamoxifen because of incomplete/slow metabolism.

- MDs use side-effect symptoms to determine efficacy of drug – may be misleading for low AND high metabolizers.

- Recent research has shown that 7–10% of women with breast cancer may not receive the full medical benefit from taking tamoxifen due to their unique genetic make-up.

- Further up to 30% of patients quit before 5 years due to bad side-effects.

- And yet…the FDA does NOT recommend genetic testing for 2D6 mutations.

- Therefore taking all this into account – why not measure the active drug circulating and be done with it!
Mass Spectrometry and Tamoxifen
Introduction

Why LC-MS/MS?

• Improved clinical utility
  • Improved sensitivity
  • Improved specificity

• Large volume laboratory
  • Automation
  • Robust

• Six sigma quality
  • Much less subjectivity
Parts of an LC/MS/MS System

Extraction
- SPE Liq/Liq
- Protein ppt.
- Dilution
- HTLC

HPLC
- Reverse phase ion-exchange;
  - no column

MS/MS
- ESI
- APCI

Data
Inside the Tandem Mass Spectrometer

Ions Enter from Nebulizer

Curtain Gas Interface

Skimmer

Q0 Ion Focusing

Orifice

Ion Selection

Parent Ion Selection

Daughter Ion Selection

Q1

Q2

Q3

Collision

Detector
Example of a patient run showing the metabolites
## Precision (% CV) Study From Assay Validation

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Level 1</th>
<th></th>
<th>Level 2</th>
<th></th>
<th>Level 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within Run</td>
<td>Total</td>
<td>Within Run</td>
<td>Total</td>
<td>Within Run</td>
<td>Total</td>
</tr>
<tr>
<td>Endoxifen</td>
<td>13.41</td>
<td>11.65</td>
<td>4.59</td>
<td>6.54</td>
<td>5.28</td>
<td>5.53</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>7.37</td>
<td>6.82</td>
<td>6.28</td>
<td>8.58</td>
<td>5.38</td>
<td>6.36</td>
</tr>
<tr>
<td>N-Desmethyl-Tamoxifen</td>
<td>5.63</td>
<td>7.53</td>
<td>6.24</td>
<td>6.68</td>
<td>3.78</td>
<td>6.81</td>
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<tr>
<td>4-Hydroxy Tamoxifen</td>
<td>12.42</td>
<td>10.36</td>
<td>3.83</td>
<td>5.44</td>
<td>4.20</td>
<td>5.89</td>
</tr>
<tr>
<td>N-Desmethyl-4’-Hydroxy Tamoxifen</td>
<td>6.25</td>
<td>8.96</td>
<td>3.57</td>
<td>8.08</td>
<td>4.79</td>
<td>8.19</td>
</tr>
<tr>
<td>4’-Hydroxy Tamoxifen</td>
<td>8.51</td>
<td>11.29</td>
<td>5.08</td>
<td>7.82</td>
<td>4.92</td>
<td>7.57</td>
</tr>
<tr>
<td>Norendoxifen</td>
<td>10.30</td>
<td>9.68</td>
<td>8.36</td>
<td>7.41</td>
<td>10.20</td>
<td>7.79</td>
</tr>
</tbody>
</table>
## Assay Characteristics and Reference Intervals for TAM and Metabolites

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LLOQ (ng/mL)</th>
<th>Observed Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoxifen</td>
<td>0.4</td>
<td>0.93-43.19</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>1.5</td>
<td>12.5-233.1</td>
</tr>
<tr>
<td>N-Desmethyltamoxifen</td>
<td>1.5</td>
<td>3.0-374.0</td>
</tr>
<tr>
<td>4-Hydroxytamoxifen</td>
<td>0.2</td>
<td>0.24-5.05</td>
</tr>
<tr>
<td>N-Desmethyl-4’-Hydroxytamoxifen</td>
<td>0.4</td>
<td>1.17-19.95</td>
</tr>
<tr>
<td>4’-Hydroxytamoxifen</td>
<td>0.4</td>
<td>0.4-6.33</td>
</tr>
<tr>
<td>Norendoxifen</td>
<td>1.2</td>
<td>&lt;7.3</td>
</tr>
</tbody>
</table>
Patient distributions and levels for the metabolites
Putting it all together
Summary

- Tamoxifen is a pro-drug and needs to be metabolized into an active form
- Many CYPs involved but 2D6 is very important
- Mutations in 2D6 may lead to low levels of Endoxifen
- Mutations may also lead to very HIGH levels of Endoxifen and Norendoxifen
- Genetic makeup is only part of the story so genetic testing not used
- Monitoring metabolites levels gives insight to individualsphenotypic response
- Allows MD to alter dosage or change therapy
- Mass spectrometry provides view of overall personal Tamoxifen phenotype
ApoE Isoform Determination by Mass Spectrometry

Part of the Alzheimer’s disease work-up
Apolipoprotein E and Alzheimer’s Disease

- Alzheimer's is a neurodegenerative disease causing memory loss, cognitive impairment, and ultimately death

- Neuronal deterioration is caused by:
  - Amyloid plaques (Aβ 40 and 42)
  - Neurofibrillary tangles (total Tau and pTau)

- Potential biomarkers for AD include:
  - Beta amyloid (Aβ) 40 and 42 peptide
  - Apolipoprotein E isoforms
  - Total tau protein
  - Phosphorylated tau
An Overview of Alzheimer’s Disease (AD)

- According to the Alzheimer’s Association, 5.4 million Americans suffer from AD
  - ~11% of people over 65
  - ~32% of people over 85
  - Approximately 200,000 people under 65 (early onset)
- Patients are screened for AD only after symptoms arise, and currently the only ways to clinically diagnose AD is by:
  - Mini-mental screening exam (MMSE)
    - Mild dementia: 20-24
    - Moderate dementia: 13-20
    - Severe dementia: <12
  - Brain imaging
  - Examination of the patients post-mortem brain
- The goal is to develop diagnostic tests that can predict AD before symptoms arise

Alzheimer’s Association, 2014
Apolipoprotein E

- Located on chromosome 19
- Three distinct alleles
  - E2, E3, E4
- Alleles are co-dominant
- Six physiologically different phenotypes:
  - E2/E2
  - E2/E3
  - E2/E4
  - E3/E3
  - E3/E4
  - E4/E4
Apolipoprotein E Function

- Found in chylomicrons and intermediate-density lipoproteins

- Produced in both peripheral tissue and neural tissue

**Peripheral Tissue**
- Produced mainly in the liver
- Mediates cholesterol metabolism
- Transports VLDL and chylomicrons

**Neural Tissue**
- Produced by astroglia and microglia cells
- In the CNS, is the predominant apolipoprotein in HDL particles
- Helps promote injury repair in the brain
- Promotes beta amyloid (Aβ) clearance
Influence of Apolipoprotein E in Neural Tissue

- Plays several roles in neural tissue development and regeneration

Joachim Herz & Uwe Beffert

*Nature Reviews Neuroscience* 1, 51-58 (October 2000)
doi:10.1038/3503622
ApoE Structure and Isoforms

- Subdivided into three regions
  1. Receptor binding region: N-terminus
  2. Flexible hinge region
  3. Lipid binding region: C-terminus

- Three Polymorphisms resulting in three isoforms
  1. ApoE2
  2. ApoE3
  3. ApoE4

- Result of two SNPs involving amino acids 112 and 158

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Liu, C.-C. et al. (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy

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Trends in Molecular Medicine 2010 16, 287-294DOI: (10.1016/j.molmed.2010.04.004)
Apolipoprotein E and Alzheimer’s Disease

- Alzheimer's is a neurodegenerative disease causing memory loss, cognitive impairment, and ultimately death

- Neuronal deterioration is caused by:
  - Amyloid plaques (Aβ 40 and 42)
  - Neurofibrillary tangles (total Tau and pTau)

- Potential biomarkers for AD include:
  - Beta amyloid (Aβ) 40 and 42 peptide
  - Apolipoprotein E isoforms
  - Total tau protein
  - Phosphorylated tau

Validated, in validation, or in development
Apolipoprotein E and Alzheimer’s Disease

- Apo E4 allele associated with a marked increase in developing Alzheimer’s disease

- Using E3/E3 as a “benchmark”, individuals with the following genotypes have an increased odds of developing AD:
  - E2/E4 = ~2.6x increase
  - E3/E4 = ~3.2x increase
  - E4/E4 = ~15x increase
  - E2/E2 & E2/E3 = ~0.6x increase
    - E2 allele protective?

- Domain interaction in ApoE4 isoform
  - Results in a “molten globule” state
  - Preferential binding of large lipoprotein particles
  - Underlying pathogenic role in AD disease
The role of apolipoprotein E4 in Alzheimer disease pathogenesis

Liu, C.-C. et al. (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy
## ApoE Quantitation and Phenotype Identification

All below steps are automated by a Hamilton Microlab Star and Performed in a 96 well plate.

<table>
<thead>
<tr>
<th>Addition of Digestion Buffer</th>
<th>Sample Addition</th>
<th>Enzyme Addition</th>
<th>Solid-Phase Extraction</th>
<th>LC-MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Calibrator Dilution</td>
<td>- Protein digestion</td>
<td>- Sample clean-up</td>
<td>- Total ApoE quantitation</td>
<td></td>
</tr>
<tr>
<td>- Linear range: CSF: 1.0-20ug/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 81 Patient Samples per plate</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>

**Calibration**

- Linear range: CSF: 1.0-20ug/mL
- 81 Patient Samples per plate

**Run Time**

- ~4 hours to run 81 patient samples
Quantitation total ApoE in CSF

CSF
Linear Range: 1-20µg/mL

Total ApoE

\[ Y = 0.0159919 + 0.086894X + 0.00452136X^2 \]

\[ R^2 = 0.9975 \]

\[ W: 1/X \]
ApoE Isoforms E2, E3, and E4

ApoE2
- Least common isoform
  - Allele frequency ~6%
- Binds poorly to cell surface receptors
- Implicated in CVD
- Decreases clearance of dietary fats

ApoE3
- "Wild type" and most common isoform
  - Allele frequency ~80%

ApoE4
- Implicated in neurological disorders, including:
  - Allele frequency ~14%
  - Alzheimer’s disease
  - Poor recovery from TBI
  - Impaired cognitive function
  - E3/E4: ~3x more likely
  - E4/E4: ~15x

ApoE2 and ApoE3
- Unique
- Shared

ApoE3 and ApoE4
- Shared
- Unique

NH3-...-COOH
Chromatogram of an Individual with ApoE2/2 Isoform
Chromatogram of an Individual with ApoE2/3 Isoform
Chromatogram of an Individual with ApoE2/4 Isoform
Chromatogram of an Individual with ApoE3/3 Isoform
Chromatogram of an Individual with ApoE3/4 Isoform
Chromatogram of an Individual with ApoE4/4 Isoform
ApoE Allele Frequency based off of 319 Individual Serum Samples by LC-MS/MS

<table>
<thead>
<tr>
<th>Allele Frequency*</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>512</td>
<td>86</td>
</tr>
<tr>
<td>% Occurrence</td>
<td>6.27%</td>
<td>80.25%</td>
<td>13.48%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1</td>
<td>30</td>
<td>7</td>
<td>210</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>% Occurrence</td>
<td>0.31%</td>
<td>9.40%</td>
<td>2.20%</td>
<td>65.83%</td>
<td>19.75%</td>
<td>2.51%</td>
</tr>
</tbody>
</table>

Isoform-specific amino acid difference

<table>
<thead>
<tr>
<th>Isoform-specific amino acid difference</th>
<th>General</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo-E2</td>
<td>112</td>
<td>158</td>
</tr>
<tr>
<td>Apo-E3</td>
<td>Cys</td>
<td>Cys</td>
</tr>
<tr>
<td>Apo-E4</td>
<td>Arg</td>
<td>Arg</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>77.9</td>
<td>59.4</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>36.7</td>
</tr>
</tbody>
</table>
Summary

- The use of MS analysis allows a cheap, fast and accurate determination of Apo-E isoforms within a plasma or CSF sample.
- From the MS data it is possible to impute the allele types present in the patient.
- There is 100% concordance with >300 samples which had been traditionally genetically sequenced.
- Allows for the identification of the alleles in the same assay in which amyloid B 42 and 40 are being also measured by MS.
- Reduces sample requirement and increases speed of TAT.
Final Thoughts

- The overall phenotype of the individual is a sum of their genotype, diet, lifestyle, disease states, medication use and more.

- Due to this, whilst the use of genotyping can identify specific mutations and inform the physician it only gives a partial view of the entire patient makeup.

- By utilizing mass spectrometry to monitor the metabolism of a drug as well as the drug levels this gives a view of what the patient’s body “sees.”

- This encompasses the genotype, lifestyle and all other modifying effects on the person which can change day to day.

- Furthermore the use of MS to impute alleles by measuring the subsequent protein is highly useful.

- May help with sequences that are difficult to sequence (e.g. G rich regions).
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