Molecular Genetic Testing in Public Health and Clinical Settings

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Outline

1. Public Health and Genetics - The higher view
2. What is driving the integration of genetic testing into practice?
3. Lessons learned: cystic fibrosis, hereditary hemochromatosis, and hereditary hearing loss
4. Future challenges and opportunities: diabetes screening, metabolic screening using molecular diagnostics, pharmacogenomics, and patient driven health care services
5. Recap - What do we know and what don't we know?

- Heart disease: 652,091
- Cancer: 559,312
- Stroke (cerebrovascular diseases): 143,579
- Chronic lower respiratory diseases: 130,933
- Accidents (unintentional injuries): 117,809
- Diabetes: 75,119
- Alzheimer's disease: 71,599
- Influenza/Pneumonia: 63,001
- Nephritis, nephrotic syndrome, and nephrosis: 43,901
- Septicemia: 34,136
Heredity / Genetic Testing / Healthcare

2nd Century AD
(Talmud - Hemophilia)

2001 AD

Public Health Services
(population-focused)

Clinical Services
(patient-focused)

Patient-Driven / Direct-to-Consumer
What is Driving the use of Molecular Genetic Testing?

1. Clinical / Public Health usefulness resulting from the Human Genome Project and other studies

2. Promise of "Genetics" and "Personalized Health Care"

3. Technology
The Technology of Molecular Genetics

Targeted mutation analysis

Sequence scanning

Sequencing

Copy number variation / Quantitative sequencing
# High-Throughput Sequencing Platforms

<table>
<thead>
<tr>
<th>Sequencing System</th>
<th>Estimated System Cost</th>
<th>Consumable cost per run</th>
<th>Gigabases ((10^9)) sequenced per run</th>
<th>Run Time per run (hours)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 Life Sciences Sequencer</td>
<td>$500,000</td>
<td>n/a</td>
<td>0.1</td>
<td>7.5 hours</td>
<td>99.5%</td>
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<tr>
<td>Illumina Genome Analyzer</td>
<td>$400,000</td>
<td>$3000</td>
<td>1.5</td>
<td>2.5 days</td>
<td>&gt;98.5%</td>
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<tr>
<td>ABI Solid™ System</td>
<td>$525,000</td>
<td>$3390</td>
<td>3</td>
<td>5-7 days</td>
<td>99.94%</td>
</tr>
<tr>
<td>Helicos Heliscope</td>
<td>n/a</td>
<td></td>
<td>7.5-10</td>
<td>3-7 days</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

*Bosch et al, J Mol Diag 2008;10:484*
### Commercial Oligonucleotide Array Platforms

<table>
<thead>
<tr>
<th>Company</th>
<th>Platform</th>
<th>Resolution</th>
<th>Probe #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Technologies</td>
<td>4 x 44K CGH</td>
<td>43 kb</td>
<td>43,000</td>
</tr>
<tr>
<td></td>
<td>1x10⁶ CGH</td>
<td>2.1 kb</td>
<td>963,000</td>
</tr>
<tr>
<td>Affymetrix</td>
<td>SNP Array 6.0</td>
<td>0.7 kb</td>
<td>906,600</td>
</tr>
<tr>
<td>Illumina</td>
<td>Human 1M-Duo</td>
<td>1.5 kb</td>
<td>1.1 x 10⁶</td>
</tr>
<tr>
<td>NimbleGen</td>
<td>385K</td>
<td>6.27 kb</td>
<td>385,000</td>
</tr>
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Molecular Genetics for Heritable Conditions in Public Health Laboratories

Screening - Identifying persons at higher risk for a medical condition.

Confirmatory Testing - follow up testing used as part of a diagnostic protocol to confirm or rule out a diagnosis for a medical condition.

THESE TERMS MAY NOT BE MUTUALLY EXCLUSIVE!
Cystic Fibrosis: Population Studies Count

1. Autosomal-recessive disease - chloride channel defect
   - high carrier frequency (1/25 and up)
   (varies according to race ethnicity)

2. Secretory defect (lungs, pancreas, etc.)
   Expected lifespan
   1960: ~ 1 year
   2000: ~ 30+ years

3. Pathology linked to mutations in CFTR gene (> 1300)

4. DNA testing is available

5. Test used for newborn screening, diagnostic testing, determining carrier status, and prenatal diagnosis
When is a mutation not a mutation: The Story of I148T

Why are population-based studies important?

The I148T mutation
- Found in affected individuals
- High prevalence

<table>
<thead>
<tr>
<th>Population</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian</td>
<td>0.7%</td>
</tr>
<tr>
<td>Canadian</td>
<td>0.2%</td>
</tr>
<tr>
<td>US Patients</td>
<td>1.1%</td>
</tr>
<tr>
<td>French</td>
<td>0.08%</td>
</tr>
<tr>
<td>French-Canadian</td>
<td>9.1%</td>
</tr>
</tbody>
</table>
When is a mutation not a mutation: The Story of I148T

42,784 patients tested
1,754 patients are carriers
113 (6.4%) patients had I148T

4,318 patients diagnosed with CF
6 patients have I148T (0.06%)

\[
\frac{6.4}{0.06} = 107 \times
\]

Not Expected!

Reason: Presence of the actual disease associated mutation:

\textbf{3199del6}

Lessons Learned

The presence of a mutation in an affected population requires additional study to establish disease association.

Problem: What about those misidentified as carriers?
Hereditary Hemochromatosis: A Screening Test Not Ready for Prime Time?

- Autosomal recessive disease caused by the accumulation of body iron
- Progression, if not treated, results in liver cirrhosis, diabetes, and death
- Diagnosis is by iron studies, quantitative phlebotomy, liver biopsy, and/or genotype
- Phlebotomy provides an effective treatment
Hereditary Hemochromatosis: A Clinical and Public Health Issue?

In 1996, two mutations were found, one of which was almost always present in affected individuals.

Many studies validated that these mutations were indeed disease associated.

**Conclusion**

A great screening test for identifying persons at risk?
WRONG! The Rest of the Story

Systematic Reviews:

- Natural history not well understood
- Iron studies are not predictive of disease progression
  (with the possible exception of high ferritin levels)
- <50% of persons with mutations in the HFE gene will become
  symptomatic - LOW PENETRANCE (and/or age of onset uncertain)

Use of the DNA test only recommended for symptomatic persons where
other exclusions are made.
Lessons Learned

Disease onset is not a given:

Our understanding of the natural history of hemochromatosis is insufficient
Genetic testing is primarily used for establishing an etiologic diagnosis.
GJB2 (Connexin 26) Newborn Screening?

1. Hearing loss - Incidence of 1-3 per 1000
   GJB2 associated sensorineural hearing loss - 14 in 100,000

2. > 400 genes known

3. Mutations in GJB2, encoding the gap junction protein, Cx26, is the most common cause of nonsyndromic prelingual sensorineural hearing loss (autosomal recessive).

4. Common mutations (e.g., 35delG - Caucasians; 167delT - Ashkenazi Jewish; 235delC/V37I - Asian)
GJB2 (Connexin 26) Newborn Screening?

1. Auditory testing does not capture every case
   - Do not know how many are missed

2. Early onset hearing loss does not always result in those harboring mutations

3. What do we know about the correlation of newborn hearing screening results and genetic testing?
   - Hispanics have significant lower association with NSHL and GJB2 than other ethnicity/races


Lager scale population-based studies are needed to establish the utility for screening.
Type 1 Diabetes - Needing an Intervention

- Autoimmune disease / In 1997, 1.6 million new cases were diagnosed in people <20 yrs old (from the National Diabetes Information Clearinghouse, NIDDK, NIH)
- Specific HLA Loci (Class II, DR / DQ) attribute to > 50% of risk
- Diabetes Autoimmunity Study in the Young (DAISY)
- When diagnosed through a screening / follow up, a less severe onset and milder clinical course ensued during the first year
  
  Barker et al Diabetes Care 2004;27:1399

It is anticipated that when an effective intervention is developed, greater emphasis will be placed upon evolving screening programs
Molecular Genetics for Heritable Conditions in Public Health Laboratories

Conditions in which a molecular confirmatory (diagnostic) follow up is indicated on an ACTion sheet (http://www.acmg.net)

- Cystic Fibrosis
- Congenital Hearing Loss
- Biotinidase Deficiency
- Galactosemia
- Amino Acidemias (e.g., PKU)
- Endocrine Disorders (e.g., congenital adrenal hyperplasia)
- Fatty Acid Disorders (e.g., LCHAD, MCAD)
- Hemoglobin Disorders (e.g., Sickle Cell / Thalassemia)
- Organic Acidemias (e.g., Glutaric Aciduria Type 2)
Pharmacogenetics / Pharmacogenomics

"Delivering the right medicine at the right dose to the right patient in a timely manner"
A Few Examples:

**Drug Selection**
HLA*B5701 - Use of Abacavir for HIV antiviral therapy

**Achieving a Maintenance Dose**
Anticoagulation therapy - CYP2C9 / VKORC1 - Warfarin

**Minimizing adverse drug reactions**
Metastatic Colon Cancer - UGT1A1 - Irinotecan
Pharmacogenomics: Should Metabolizer Status be Determined During the Newborn Screen or other Public Health Initiative?

- Do we know enough to offer this?
- Is this a "Public Health" issue?
- Technologically feasible?
- How should the test results be managed?
- Utility?
Patient-Driven and Direct-to-Consumer Health Care Service

Evidence-Based

Trial and Error (Experience / Expert opinion)

MANAGEMENT DECISION?

Direct Access-to-Consumer Genetic Testing Companies
(~ 38 as of 2/3/2009 - Johns Hopkins Genetics and Public Policy Center
http://www.dnapolicy.org/resources/DTCcompanieslist.pdf)
Summary

• Molecular genetics is applicable to clinical and public health practice

• Population studies count (cystic fibrosis)

• Using genetics to predict disease requires careful consideration (hemochromatosis)

• Having an effective intervention and establishing benefits is important (diabetes / GJB2 screening)

• The public desires and has direct access to health-related information; judging what is credible is essential for appropriate health care decision making.
Thank you! Questions?

Good!

MMMMM