Genetic Diseases Due to Single Gene Defects: Case Studies

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Why a lecture (or two) on Molecular Pathology

Paper from NEJM. An overweight patient shows up to your office with analysis of their entire genome sequenced. Pt will cite data about chances of a particular disease and will ask you, "what does this mean?" Your challenge will be to know how to respond.

Letting the Genome out of the Bottle — Will We Get Our Wish?
David J. Hunter, M.B., B.S., Sc.D., M.P.H., Muin J. Khoury, M.D., Ph.D., and Jeffrey M. Drazen, M.D.

It may happen soon. A patient, perhaps one you have known for years, who is overweight and does not exercise regularly, shows up in your office with an analysis of his whole genome at multiple single-nucleotide polymorphisms (SNPs). His children, who were concerned about his health, spent $1,000 to give him the analysis as a holiday gift. The test report states that his genomic profile is types. These studies rely on microarrays that can assess 300,000 or more SNPs in each DNA sample; researchers use these microarrays to examine interperson- nal differences in inherited genetic The test undergone by the patient described above is one of the products of this new knowledge.

As of November 2007, two companies have made available direct-to-consumer “personal genome services” (www.23andme.com) or “gene profiles” (www.decodeme.com) that rely on the same arrays of 500,000 to 1 million SNPs used in genomewide association studies. A third com-
Why a lecture (or two) on Molecular Pathology

Companies exist that will seq your genome. These companies don't provide genetic interpretation. That's your job.
Why a lecture (or two) on Molecular Pathology

Lecture Objective - Understand the fundamental concepts underlying the molecular nature of disease and the utility, practice and pitfalls of molecular diagnostic testing

(at least as well as your patients...)
Resources

A Brief Word on the Chemical Nature and Structure of DNA

1962: Nobel Prize for the Discovery Of the Structure of DNA
James Watson, Francis Crick: DNA is a double helix composed of anti-parallel strands with bases paired on the inside and a phosphates backbone on the outside.
The Central Dogma

DNA → Transcription → RNA → Processing Introns / PolyA → mRNA → Translation 3 base code → Protein

MUTATIONS

Our focus today.

Postranslational Modification
What Diseases Are Associated with Specific Mutations?

All inherited diseases
CF, HFE, GSD, ETC
Tumor syndromes (FAP, HNPCC, ETC)

All Neoplasms
Associated with acquired mutations.

A lot of diseases that you wouldn’t expect.
Type II diabetes, prostate cancer, risk of heart disease, any many others.

The birth of molecular diagnostics
Linus Pauling describes first molecular abnormality associated with a disease process.

Nobel prize in chemistry 1954
"for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances"
Nobel peace prize 1962

Molecular diagnostics and the molecular basis of disease was discovered before the discovery of DNA structure. Linus Pauling showed that hemoglobin from a person with sickle cell anemia and a normal person travelled differently under an electrical gradient. Furthermore, the trait was heritable. This linked inheritability with molecular abnormality. It was a big deal.
What Diseases Are Associated with Specific Mutations?

All inherited diseases
CF, HFE, GSD, ETC
Tumor syndromes (FAP, HNPCC, ETC)
All Neoplasms
A lot of diseases that you wouldn’t expect.
Type II diabetes, prostate cancer, risk of heart disease, any many others.

The Genetic Information Nondiscrimination Act of 2008 (GINA) was enacted on May 21, 2008 (Pub. L. 110-233). Title I of GINA amends the Employee Retirement Income Security Act of 1974 (ERISA), the Public Health Service Act (PHS Act), the Internal Revenue Code of 1986 (Code), and the Social Security Act (SSA) to prohibit discrimination in health coverage based on genetic information.

Law passed in 2008. Basically says that you can't discriminate people based on their genetic information (like sex or race).
How are Genes Mutated?

DNA damaging agents (UV, chemical)

Errors in replication or repair

Inherited (germ line) mutations

**Point mutations**: Mutation of a single base pair. (silent vs. missense vs. nonsense)

**Deletions**: loss of one or more base pairs from a gene sequence. (including frame-shift mutations)

**Insertions**: Insertion of one or more base pairs into a gene sequence. (including frame-shift mutations)

**Repeat Expansions**: multiplication of tri-nucleotide regions within gene regulatory regions.

Repeat sequences can become unstable during some disease processes.
Inherited Mutations

Autosomal Dominant
Autosomal Recessive
X-Linked
  Recessive
  Dominant
Mitochondrial Mutations

Mutations can be any of these.
Autosomal dominant inheritance

Pop Quiz

50% of children are affected by disease
Common Autosomal Dominant Inherited Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial Hypercholesterolemia</td>
<td>1 / 500</td>
</tr>
<tr>
<td>Adult Polycystic Kidney Disease</td>
<td>1 / 1,000</td>
</tr>
<tr>
<td>Hereditary Spherocytosis</td>
<td>1 / 5,000</td>
</tr>
<tr>
<td>Neurofibromatosis Type 1</td>
<td>1 / 3,500</td>
</tr>
<tr>
<td>Familial Adenomatous Polyposis</td>
<td>1 / 10,000</td>
</tr>
<tr>
<td>Ehlers-Danlos Syndrome</td>
<td>1 / 5,000</td>
</tr>
<tr>
<td>Marfan Syndrome</td>
<td>1 / 10,000</td>
</tr>
<tr>
<td>Huntington Disease</td>
<td>1 / 15,000</td>
</tr>
<tr>
<td>HNPCC</td>
<td>3% (of Colon Cancer)</td>
</tr>
</tbody>
</table>

Not enough time to talk about all autosomal dominant diseases. Expects you to read about these on your own. Good idea, since Step 1 loves this stuff.
Autosomal recessive inheritance

25% of offspring are homozygotes (affected by disease)
50% are heterozygotes (carriers)
25% are normal

Pop Quiz

If disease is lethal what is the probability that this person is a carrier? 66%. Why? Because those who are alive can only be heterozygotes (carriers) or normals. They have 50% and 25% distribution respectively and so this turns into 2/3 and 1/3 respectively.
Autosomal Recessive Inherited Diseases

- Hemochromatosis 1 / 200
- Cystic Fibrosis 1 / 2,500
- Phenylketonuria 1 / 12,000
- Gaucher disease 1 / 1,000 (Ashkenazi Jews)
- Tay-Sachs disease 1 / 4,000 (Ashkenazi Jews)
- Von Gierke disease 1 / 100,000
- Galactosemia 1 / 18,000 to 1 / 100,000

Autosomal recessive mutations are quite common in populations. Hemochromatosis is very common. You should know these because you'll see it in practice.
X-linked recessive inheritance

In this pedigree: If male 50% chance, if female 50% carrier, 0% affected
### X-linked recessive inheritance

<table>
<thead>
<tr>
<th>Condition</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragile X Syndrome</td>
<td>1 / 3,000</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>1 / 5,000</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>1 / 40,000</td>
</tr>
<tr>
<td>Duchenne Muscular Distrophy</td>
<td>1 / 5,000</td>
</tr>
<tr>
<td>Chronic Granulomatous Disease</td>
<td>1 / 200,000</td>
</tr>
<tr>
<td>Lesch-Nyham Syndrome</td>
<td>1 / 400,000</td>
</tr>
<tr>
<td>Wiskott-Aldrich Syndrome</td>
<td>1 / 1,000,000</td>
</tr>
</tbody>
</table>

Many of these. "Look them up on your own."
Single Nucleotide Polymorphisms

Single nucleotide changes in genes that subtly affect function.

Accounts for differences in individuals.

- Factor V leiden and clotting risk
- Compliment Factor H and AMD
- TCF7 polymorphisms and diabetes
- CYP2c9 polymorphisms and wafarin metabolism

While these don't cause overt disease, they affect our response to environment.
Medical Genetics: Case #1

60 year old male with recent onset diabetes mellitus, hepatomegaly, skin pigmentation, cardiac arrhythmias, arthritis and loss of libido.

Liver CT concerning for cirrhosis, biopsy performed.
This is a liver biopsy using special stain that shows fibrous tissue (trichrome stain). You can see bands of fibrous tissue. This shows cirrhosis.

Colloidal iron stain (blue). Lots of iron in this liver --> diagnosis is hemachromatosis.

H&E stain. Brown speckling in hepatocytes and bile duct epithelium.

Diagnosis?
TRUE / FALSE?

1. This disease has both an inherited form and an acquired form.
   - You can acquire it by increased in intake of iron (usually from multiple transfusions).

2. The inherited form of this disease usually become evident after the age of 40.

3. The inherited form has a male predominance (5:1).

4. The inherited form is an autosomal recessive disease.

5. The disease frequency (homozygotes) in individuals of Northern European descent is 1 in 200 (0.5%) with an allele frequency of 10%.

6. Carriers usually don’t manifest symptoms.

7. Serum iron and ferritin are both elevated in this disease process.

8. Associated with a 200x increase in HCC
   - HCC = hepatocellular carcinoma

People with hemachromatosis have increased iron uptake in gut, which leads to increased levels of serum iron and ferritin. Menses causes incidence to go down in women. Most people die without knowing they have the disease.
TRUE / FALSE?
1. This disease has both an **inherited** form and an acquired form.
2. The inherited form of this disease usually become evident after the age of 40.
3. The inherited form has a **male** predominance (5:1).
4. The inherited form is an **autosomal recessive** disease.
5. The disease frequency (homozygotes) in individuals of Northern European descent is 1 in 200 (0.5%) with an allele frequency of **10%**.
6. Carriers usually don’t manifest symptoms.
7. Serum iron and ferritin are both elevated in this disease process.
8. Associated with a **200x increase** in HCC
Medical Genetics: Hemochromatosis

TRUE/FALSE

1. An autosomal recessive disease of iron metabolism resulting from a mutation of the HLA-H gene (HFE) (class I MHC like molecule) on chromosome 6.

2. A single genetic mutation accounts for > 95% of cases: Cysteine 282 - Tyrosine.  

3. Can be diagnosed by a simple PCR-based test on peripheral blood.

4. The disease penetrance approaches 20%

---

T 1. True
T 2. True
T 3. True
T 4. True
Medical Genetics: Hemochromatosis

TRUE/FALSE

1. An autosomal recessive disease of iron metabolism resulting from a mutation of the HLA-H gene (HFE) (class I MHC like molecule) on chromosome 6.

2. A single genetic mutation accounts for > 95% of cases: Cysteine 282 - Tyrosine. (Also H63D and S65C in HFE, 19q13, and Hemojuvulin 1q21)

3. Can be diagnosed by a simple PCR-based test on peripheral blood.

4. The disease penetrance approaches 20%
This study looks at transferrin saturation of people with different genotypes.

Not much of an effect.
KEY CONCEPT: Polymorphism vs. Mutation

Some variants are simply polymorphisms rather than disease causing mutations.
# Medical Genetics: Hemochromatosis

<table>
<thead>
<tr>
<th>Race or Ethnic Group</th>
<th>Total No. of Participants</th>
<th>C282Y/C282Y</th>
<th>C282Y/H63D</th>
<th>H63D/H63D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Prevalence (95% CI)</td>
<td>No.</td>
</tr>
<tr>
<td>White</td>
<td>44,082</td>
<td>281</td>
<td>0.44 (0.42–0.47)</td>
<td>908</td>
</tr>
<tr>
<td>Native American</td>
<td>648</td>
<td>1</td>
<td>0.11 (0.061–0.20)</td>
<td>7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>12,459</td>
<td>7</td>
<td>0.027 (0.022–0.032)</td>
<td>48</td>
</tr>
<tr>
<td>Black</td>
<td>27,124</td>
<td>4</td>
<td>0.014 (0.012–0.017)</td>
<td>35</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>698</td>
<td>0</td>
<td>0.012 (0.0043–0.032)</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>12,772</td>
<td>0</td>
<td>0.000039 (0.000015–0.000010)</td>
<td>0</td>
</tr>
<tr>
<td>Multiple/unknown</td>
<td>1,928</td>
<td>6</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>All</td>
<td>99,711</td>
<td>299</td>
<td>—</td>
<td>1017</td>
</tr>
</tbody>
</table>

Low prevalence in non-whites.
Medical Genetics: Hemochromatosis

60 year old male with recent onset diabetes mellitus (75%), Cirrhosis (100%), skin pigmentation (75%), cardiac arrhythmias, arthritis and loss of libido.

**Classic Triad**

**Diagnosis in stages**

- Elevated total serum iron and ferritin,
- Liver biopsy with quantitative iron measurement (> 10,000 ug/gm).
- Symptoms begin at 20 gm of storage iron.
- Molecular analysis of HFE
1 year old child with intestinal malabsorption, poor weight gain, recurrent and persistent lung infections. A chloride sweat test shows elevated sweat electrolyte concentrations.

Cystic fibrosis.
Medical Genetics: Case #2

Tissue? Finding?

Lung tissue. Abundant chronic inflammation. Big ball of mucus.
Pancreas. Islets of Langerhans are missing the acini. CF was initially described as cystic change and fibrosis of the pancreas.
TRUE/FALSE?  
1. Typical symptoms of this disease include bronchiectasis, pancreatic insufficiency, male infertility and hepatic biliary cirrhosis.  
2. Results from mutations in the CFTR gene, a 24 exon, 1480 AA transmembrane chloride ion channel at 7q31  
3. 10’s to 100’s of mutations accounts for 95% of affected individuals: delta F508 is the most common in European descent (but still ONLY 65%)  
4. The disease is inherited in an autosomal recessive fashion.  
5. This is the most common lethal genetic disease in the US affecting 1:1500 to 1:4000 live births with a carrier frequency of 2 - 4%.  
6. Carrier screening is recommended by the ACMG for all women who are pregnant or planning pregnancy
TRUE/FALSE?

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Cystic Fibrosis USA mutation frequencies

Bobadilla et al., (2002)
There are different classes of mutations (in red box).

CFTR – Classes of Mutations

- 1. Absence of mRNA synthesis
- 2. Defective protein maturation / folding
- 3. Disordered regulation (ATP hydrolysis)
- 4. Defective chloride conductance
- 5. Defective splicing or reduced expression
- 6. Increased turnover

Figure 1. Categories of CFTR Mutations.
Classes of defects in the CFTR gene include the absence of synthesis (class I), defective protein maturation and premature degradation (class II), disordered regulation, such as disturbed ATP binding and hydrolysis (class III), defective chloride conductance or channel gating (class IV), a reduced number of CFTR transcripts due to a promoter or splicing abnormality (class V), and accelerated turnover from the cell surface (class VI).
CFTR – Classes of Mutations

- 1. Absence of mRNA synthesis
- 2. Defective protein maturation/folding
- 3. Disordered regulation (ATP hydrolysis)
- 4. Defective chloride conductance
- 5. Defective splicing or reduced expression
- 6. Increased turnover

KEY CONCEPT: The Severity of Disease Can Vary Depending on the Type of Mutation

Rowe et al. NEJM 2005
Cystic Fibrosis USA mutation frequencies

Babadilla et al., (2002)

KEY CONCEPT: Residual Risk
Cystic Fibrosis USA mutation frequencies

Bobadilla et al., (2002)

Ethnic background dictates residual risk. You must test mutations appropriate for the target population (ethnic group). Not to do so would be a disservice to your patient.
Coagulation: Case #3

• 25 year old female with right lower extremity pain and swelling. Imaging studies reveal a deep vein thrombosis. She is otherwise healthy and her only risk factor for thrombosis is oral contraception.
Coagulation: Case #3

TRUE/FALSE?

1. A mutation in Factor V can be found in approximately 20% of patients with this clinical picture. (Glu 506 Arg)
2. The population (European descent) frequency of this allele is 3-4%.
3. Hypercoagulability due to Factor V mutations is autosomal dominant.
4. This mutation inhibits the ability of protein C to inactivate factor V.
5. This mutation increases the risk of spontaneous thrombosis 2-4 fold. 1% chance / year after 60.
6. A mutation in Prothrombin (G20210A, 3’UTR) can be found in 4-8% of these patients.

All are true. Half of you factor V is relatively resistant to the actions of protein C. A mutation in UTR of prothrombin can affect the stability of the mRNA.
TRUE/FALSE?

1. A mutation in Factor V can be found in approximately 20% of patients with this clinical picture. (Glu 506 Arg)
2. The population (European descent) frequency of this allele is 3-4%.
3. Hypercoagulability due to Factor V mutations is autosomal dominant. Homozygotes have further increased risk.
4. This mutation inhibits the ability of protein C to inactivate factor V.
5. This mutation increases the risk of spontaneous thrombosis 2-4 fold. 1% chance / year after 60.
6. A mutation in Prothrombin (G20210A, 3’UTR) can be found in 4-8% of patients with this clinical picture.
KEY CONCEPT:
The clotting cascade is big and confusing.
Coagulation: Factor V Leiden

Clotting cascade. The point: “the clotting cascade is big and confusing.” Mutations in a lot of these proteins can cause problems (Hemophilia for example).
An Aside: Coagulation + Pharmacogenomics: Warfarin Metabolism

• **Warfarin - background**
  
  – blocks vitamin K dependent clotting enzymes by inhibiting vitamin K epoxide reductase VKORC1.
    • Gama-glutamylcarboxylation of II, VII, IX and X (assay therapeutic effects through changes in INR)
  
  – Very commonly used drug for long term anticoagulation to prevent thrombo-embolic events
    • 20 million prescriptions / year to 1 million patients.
    • 1-2% chance of a major bleed
    • Estimated 0.1% mortality

Read this.
Coagulation + Pharmacogenomics: Warfarin Metabolism

• Warfarin - background
  – Metabolized by a hepatic microsomal enzyme
    • CYP2C9 (p450 enzyme)
    • Two common polymorphisms are present in CYP2C9
      – CYP2C9*1 – wt allele
      – CYP2C9*2 – R144C – 30% decrease in activity, 11% population frequency
      – CYP2C9*3 – I359L – 80% decrease in activity, 7% population frequency.

3 types of alleles for CYP2C9. If you have CYP2C9*3 then you need lower dose of warfarin because you metabolize it slower.
Coagulation + Pharmacogenomics: Warfarin Metabolism

• Warfarin - background
  – Inhibits VKORC1

  • Two common Genomic variants of VKORC1
    – -1639 G>A (promoter polymorphism)
      » GG 25%, GA 56%, AA 19%
    – 1173 C>T (Intron 1 polymorphism)
      » C allele frequency is 58%
    – Both vary significantly in different populations

Vitamin K Epoxide Reductase --> the target of warfarin

Alleles that cause higher expression of gene require higher doses.
Coagulation + Pharmacogenomics: Warfarin Metabolism

• Warfarin – the data

<table>
<thead>
<tr>
<th>Genotype</th>
<th>*1/*1</th>
<th>*1/*2</th>
<th>*1/*3</th>
<th>*2/*2</th>
<th>*2/*3</th>
<th>*3/*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>127</td>
<td>28</td>
<td>18</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Daily maintenance warfarin dose, mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.63 (2.56)</td>
<td>4.88 (2.57)</td>
<td>3.32 (0.94)</td>
<td>4.07 (1.48)†</td>
<td>2.34 (0.35)</td>
<td>1.60 (0.81)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>5.27 (3.93-7.14)</td>
<td>4.64 (3.61-5.29)</td>
<td>2.92 (2.50-3.93)</td>
<td>3.86 (2.50-4.00)</td>
<td>2.32 (2.00-2.70)</td>
<td>1.61 (1.14-1.96)</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis χ² = 37.348; P<.001. IQR indicates interquartile range.†Mean doses (mg) for the 4 patients were 2.50, 3.71, 4.00, and 6.07. The mean of these is 4.07; however, the 75th percentile is 4.00. The patient with the mean daily dose of 6.07 had a prosthetic valve and experienced a serious bleeding event. This reflects the potential skewness that data from patients with prosthetic valves can introduce to small samples and reflects the range of maintenance doses that can occur in a clinical setting. An analysis was performed in which 12 patients having prosthetic valves with a higher target international normalized ratio range (2.5-3.5) were excluded; the effect on hazard ratio estimates was trivial (see text).
Coagulation + Pharmacogenomics: Warfarin Metabolism

• Warfarin – the data

Higashi et al., (2002) JAMA

I'm going to quote him verbatim: "If we look at the time to therapeutic INR. The WT allele gets to a therapeutic INR faster than the mutant allele. And you can see that the time to the first above range INR is sooner for the mutant than the WT allele. So they require a smaller dose and they also have more life threatening bleeding events. If you have *3 allele you have more life threatening bleeding events than if you have the *1 allele."
Coagulation + Pharmacogenomics: Warfarin Metabolism

- Warfarin – the data

Sconce et al., (2005) Blood
Coagulation + Pharmacogenomics: Warfarin Metabolism

• Warfarin – the Question: Should everyone starting Warfarin be tested?

Regression Analysis to get:
Dose = 0.628 – 0.0135 (Age) – 0.240 (CYP*2) – 0.370 (CYP*3) – 0.241(VKOR) + 0.0162 (Height)
(VKOR = 1 for GG, 2 for GA and 3 for AA)

Sconce et al., (2005) Blood

This equation allows you to calculate proper dosage for patients. Genotype and then plug in values for alleles.

So should you genotype patients? Instructions of warfarin packet says you should but there isn't any data that shows that if you dose based on genotype, patients won't undergo adverse outcomes. Rarely done.
Medical Genetics: Case #4

4 year old boy who is behind in his developmental milestones, has a long face, large mandible, large everted ears. Similar findings in his older brother. His older sisters are apparently normal.

1. Probable Diagnosis?