Lecture Materials from the TRIG Working Group

Training Residents in Genomics

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All the lectures contained in this booklet are available online at [www.pathologytraining.org](http://www.pathologytraining.org). Production of these materials made possible by The Intersociety Council for Pathology Information, Inc.
“It is time to get serious about genomics education for all health care professionals,” wrote Dr. Eric Green, the director of the National Human Genome Research Institute and a pathologist by training, in a recent Commentary in the Journal of the American Medical Association.\(^1\) Indeed, a survey conducted by the (Pathology Residency) Program Directors Section (PRODS) of the Association of Pathology Chairs (APC) in the spring of 2010 demonstrated that approximately 70% of pathology residency programs do not have training in genomics. A coordinated effort to meet the genomics education challenge for anatomic pathology and laboratory medicine began in 2010.\(^2,3\) The Training Residents in Genomics (TRIG) Working Group was established in the summer of 2010 to facilitate the education of pathology residents in genomics and consists of experts in molecular pathology, genetic counseling and medical education.

One of the first goals of the TRIG Working Group was to create four lectures that can be used by training programs to supplement an already existing molecular pathology curriculum for residents. The lectures cover genomic methods, clinical interpretation of genomic testing and communicating and reporting genomic test results to other clinicians and patients. The lectures are presented in this booklet as PowerPoint presentations and will also be freely available (with added notes) online (www.pathologytraining.org) on the website of the Intersociety Council for Pathology Information (ICPI), which also publishes the annual Directory of Pathology Training Programs. For assistance with accessing the online materials, please contact icpi@asip.org. The TRIG Working Group thanks the American Society for Clinical Pathology for administrative and design support and ICPI for providing funding to produce and print this booklet.

Please note that the lectures in this booklet and the online materials have not been specifically endorsed by any of the cooperating organizations listed below. They are offered in the hope that they will provide a useful initial introduction to the subject, be helpful in designing a genomic pathology curriculum for graduate training programs, and noting that genomic pathology will continue to evolve with the need to continuously update teaching materials. For your convenience, specific references to content in the four lectures are included on the pages that follow.

On behalf of the TRIG Working Group,
Richard L. Haspel, MD, PhD
Beth Israel Deaconess Medical Center

References:
Training Residents in Genomics (TRIG) Working Group

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The following organizations have cooperated with the TRIG Working Group:
Academy of Clinical Laboratory Physicians and Scientists (ACLPS)
American Society for Clinical Pathology (ASCP)
American Society for Investigative Pathology (ASIP)
Association for Molecular Pathology (AMP)
Association of Pathology Chairs (APC)
  Program Directors Section (PRODS)
  Undergraduate Medical Educators Section (UMEDS)
College of American Pathologists (CAP)
Intersociety Council for Pathology Information (ICPI)
National Coalition for Health Professional Education in Genetics (NCHPEG)
National Society of Genetic Counselors (NSGC)
United States and Canadian Academy of Pathologists (USCAP)

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Lucy Beck

ASIP Staff:
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TRIG Working Group Lecture References

Lecture I: Genomic Pathology: An Introduction
Authors: Richard L. Haspel, MD, PhD; Mark S. Boguski, MD, PhD


Lecture II: Genomic Methods
Authors: Dennis P. Wall, PhD; Frederic G. Barr, MD, PhD; Debra G.B. Leonard, MD, PhD

14. Online Resources
   • Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)
   • International HapMap project (http://hapmap.ncbi.nlm.nih.gov)
   • Human genome mutation database (http://www.hgvs.org/dblist/glsdb.html)
   • PharmGKB (http://www.pharmgkb.org)

**Lecture III: Interpreting Genomic Information for Clinical Care**
Authors: Richard L. Haspel, MD, PhD; Karen L. Kaul, MD, PhD; Henry M. Rinder, MD


**Lecture IV: Genomic Medicine: Communicating with the Patient**

Authors: Julianne O’Daniel, MS, CGC; Joan Scott, MS, CGC; Elizabeth Varga, MS, CGC; VO Speights, DO; Erynn Gordon, MS, CGC

10. UT Southwestern Medical Center. Cancer Gene. [http://www8.utsouthwestern.edu/utsw/cda/dept47834/files/73815.html](http://www8.utsouthwestern.edu/utsw/cda/dept47834/files/73815.html); accessed 2/10/12.
11. Online Resources
   - American Board of Genetic Counseling ([www.abgc.net](http://www.abgc.net))
   - American Board of Medical Genetics ([www.abmg.org](http://www.abmg.org))
   - National Coalition for Health Professional Education in Genetics ([www.nchpeg.org](http://www.nchpeg.org))
   - American College of Medical Genetics ([www.acmg.org](http://www.acmg.org))
   - National Society of Genetic Counselors ([www.nsgc.org](http://www.nsgc.org))
Lecture I:
Genomic Pathology: An Introduction

Richard L. Haspel, MD, PhD
Mark S. Boguski, MD, PhD

$2,700,000,000
1990-2003

What do I get for $999?
You get access to your raw data of 50 million DNA bases at high quality (80X coverage). You will have access to new tools and content as they are developed, to take full advantage of your exome sequence data. Most excitingly, you'll be a trailblazer, one of the first people on the planet to know their personal exome sequence!

Coming to a clinic near you…
Based on personal experience at Stanford University Hospital & Clinics…patients have already begun to arrive at their physician's office with 23andMe reports in hand seeking expert medical advice.

Definitions
What is a human genome?
• A “whole genome” consists of ~3 gigabytes (3 billion “base pairs”) of DNA distributed unequally among 46 chromosomes (diploid genome).
• Approximately 98% of the this DNA is “intergenic” (literally “between genes”), does not encode proteins and is of unknown medical relevance.

What is a human exome?
• Exome refers to the 2% subset of the whole genome that encompasses our ~22,000 pairs of genes.
• Because each gene, on average, is composed of 8 protein-encoding segments (“exons”), an exome corresponds to 8 x 22,000 = 176,000 segments of DNA.

What is a human transcriptome (a.k.a. gene expression profile)?
• Transcriptome refers collectively to all of the “expressed” RNA “transcripts” of genes based on the “central dogma” of molecular biology, i.e. DNA -> RNA -> protein.
• A transcriptome reflects what a cell is doing at a particular point in time (molecular phenotype) as opposed to what it is capable of doing (genotype).
Genomic Testing as a Universal Diagnostic

Natural history of disease, response to therapy
Surgical pathology
Medical genetics, preventive medicine
Pharmacogenomics
Molecular mechanisms of disease

Why Pathologists? We have access, we know testing

Physician sends sample to Pathology (blood/tissue)
Access to patient’s genome

Pathologists
Personalized Risk Prediction, Medication Dosing, Diagnosis/Prognosis

Genetic Counselors
Medical Geneticists

“[a patient’s genome] is just another lab value.”

-- D.P. Dimmock
ACMG, Vancouver
18 March 2011

Sample swaps at 23andMe: a cautionary tale
By Daniel MacArthur | June 7, 2010 | 6:00 a.m.

Pathologists in Familiar Role

Pathologists

Already considering workflow

What will be the pathology report of the future?

• Ethical issues
• Informed consent
• Integrated data interpretation
Multidisciplinary Teams Needed

- Modern medical technology and practice requires a multidisciplinary approach to medical practice.
- Greater interaction needed between Pathologists, Genetic Counselors and Medical Geneticists

Training Residents in Genomics (TRIG) Working Group

What we will cover today:

- Review present and future of genomic testing:
  - Methods
    - Single gene
    - Gene chips
    - Whole-genome/exome/transcriptome sequencing
  - Clinical utility
    - Prognosis/diagnosis
    - Risk prediction
  - Communicating with the patient
    - Single gene
    - Genomic testing
Lecture II: Genomic Methods

Dennis P. Wall, PhD  
Frederick G. Barr, MD, PhD  
Deborah G.B. Leonard, MD, PhD

Why Pathologists? We have access, we know testing

Physician sends sample to Pathology (blood/tissue)
Access to patient’s genome
Personalized Risk Prediction, Medication Dosing, Diagnosis/Prognosis
Just another laboratory test

The path to genomic medicine

Pathologists

Access to patient’s genome

Sample Collection
Testing: Sequencing, Gene chips
Analysis

What we will cover today:

• Types of genetic alterations
• Current and future molecular testing methods
  – Cytogenetics, in situ hybridization, PCR
  – Gene chips
    • Genotyping
    • Expression profiling
    • Copy number variation
  – Next generation sequencing (NGS)
• Genotyping
• Expression profiling
• Copy number variation

DNA alterations – the small stuff

Point mutation
CCTCAGGAG → CCTGTGGAG
Example: hemoglobin, beta – sickle cell disease

Deletion/Insertion
GAATTAGAGAAGCA → GAAGCA
Example: epidermal growth factor receptor – lung cancer

Repeat alteration
TTCAG...[CAG]6...CAGCAA
TTCCAG...[CAG]6...CAGCAA
Example: huntingtin – Huntington disease

DNA alterations – the bigger stuff

Example: 22q11.2 region – DiGeorge syndrome

Example: 17q21.1 (ERBB2) – Breast cancer

Example: t(11;22)(q24;q12) – Ewing’s sarcoma
Previous strategies to detect DNA alterations

Cytogenetics:
Large indels, amplification, translocations

In situ hybridization:
large indels, amplification, translocations

EGFR amplification in glioblastoma

What we will cover today:

• Types of genetic alterations
• Current and future molecular testing methods
  - Cytogenetics, in situ hybridization, PCR
  - Gene chips
    - Genotyping
    - Expression profiling
    - Copy number variation
  - Next generation sequencing (NGS)
    - Whole genome
    - Transcriptome

DNA microarray - the basics

• Purpose: multiple simultaneous measurements by hybridization of labeled probe
• DNA elements may be:
  - Oligonucleotides
  - cDNA’s
  - Large insert genomic clones
• Microarray is generated by:
  - Printing
  - Synthesis

Microarray technologies

Organization of a DNA microarray
Hybridization of a labeled probe to the microarray

Detection of hybridization on microarray

Hybridization intensities on DNA microarray following laser scanning

Overview of SNP array technology

Microarray Applications

- DNA analysis
  - Polymorphism/mutation detection – e.g. Disease susceptibility testing
  - Drug efficacy/sensitivity testing
  - Copy number detection (comparative genomic hybridization) – e.g. Constitutional or cancer karyotyping
  - Bacterial DNA – e.g. Identification and speciation

- RNA analysis
  - Expression profiling – e.g. Breast cancer prognosis
  - Cancer of unknown primary origin

Genome-wide association studies of breast cancer microarray with 317,139 SNP’s
Genotype calling

Hybridization intensities translated into genotypes
Large SNP numbers requires automated procedure
Recent algorithms – clustering/pooling strategies
  • Raw hybridization intensities normalized
  • Information combined across different samples at each SNP
  • Assign genotypes to entire clusters
  • For each sample, estimate probability of each of three genotype calls at each SNP
  • Genotype assigned based on defined threshold of probability
  • Missing genotypes dependent on algorithm & threshold used

Genotyping - Limitations & quality control

• Accuracy of algorithm
  – Depends on number of samples in each cluster
  – Prone to errors for small number of samples or SNP's with rare alleles
• High rates of missing genotypes:
  – Array problems – plating/synthesis issue
  – Poor quality DNA – degradation
  – Hybridization failure
  – Differential performance between SNP’s
• Excess heterozygosity - sample contamination?

Expression profiling: challenges and limitations

Biological
  • Dynamic & complex nature of gene expression
  • Heterogeneous nature of tissue samples
  • Variation in RNA quality

Technological
  • Reproducibility across microarray platforms
  • Selection of probes – dependence on binding efficiency
  • Controlling for technical variability

Statistical/bioinformatic
  • Adequate experimental design
  • Normalization to remove variability among chips
  • Multiple testing correction
  • Validation of results

Copy number variation: Comparative genomic hybridization

Just another laboratory test
Constitutional genomic imbalances detected by copy number arrays

10.9 Mb deletion at 7q11

7.2 Mb duplication on 11q


Copy number - Limitations & quality control

Artifacts may be caused by:

- GC content
  - Wavy patterns correlate with GC content
  - Algorithms developed to remove waviness

- DNA sample quantity and quality
  - Can impact on level of signal noise and false positive rate
  - Whole genome amplification associated with signal noise

- Sample composition
  - In cancer studies, normal cells dilute cancer aberrations
  - Tumor heterogeneity will also affect copy number

What we will cover today:

- Types of genetic alterations
- Current and future genetic test methods
  - Cytogenetics, in situ hybridization, PCR
  - Gene chips
    - Gene expression
    - Copy number variation
  - Next generation sequencing (NGS)
    - Whole genome
    - Transcriptome

Just another laboratory test

Cancer Treatment: NGS in AML

Use of Whole-Genome Sequencing to Diagnose a Cryptic Fusion Oncogene

Welch JS, et al. JAMA, 2011;305, 1577

Case History

- 39 year old female with APML by morphology
- Cytogenetics and RT-PCR unable to detect PML-RAR fusion
- Clinical question: Treat with ATRA versus allogeneic stem cell transplant

Methods/Results

- Paired-end NGS sequencing

- Result: Cytogenetically cryptic event: novel fusion protein

- Took 7 weeks
77-kilobase segment from Chr. 15 was inserted en bloc into the second intron of the gene RARA on Chr. 17.

Workflow

- Image processing and base calling
- Alignment to reference genome
- Detection of genetic variation (SNPs, Indels, SV)
- Linking variants to biological information

Overview of Paired End Sequencing

- Short Insert
- Adapter Ligated
- Randomly Sheared DNA
- Annealed to Surface
- Ligated and Synthesized
- Sequenced
- Sequencing done with labeled NTPs and massively parallel

Short read output format

```
@ML:9222-3:1-2:05460/2
GGAGGACACAGGAGAACAGCCTCACTCCCC
+ML:9222-3:1-2:05460/2
aaccttactttctactgtcagactacaagctcagcc
+ML:9222-3:1-2:05460/2
aaccttactttctactgtcagactacaagctcaggg`
```

Measuring Accuracy

- Phred is a program that assigns a quality score to each base in a sequence. These scores can then be used to trim bad data from the ends, and to determine how good an overlap actually is.
- Phred scores are logarithmically related to the probability of an error: a score of 10 means a 10% error probability; 20 means a 1% chance, 30 means a 0.1% chance, etc.
  
  - A score of 20 is generally considered the minimum acceptable score.
Workflow

Raw Data Analysis
Image processing and base calling

Whole Genome Mapping
Alignment to reference genome

Variant Calling
Detection of genetic variation (SNPs, Indels, SV)

Annotation
Linking variants to biological information

Alignment/Mapping

Alignment approaches

<table>
<thead>
<tr>
<th>Aligner</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowtie</td>
<td>Fast, memory-efficient short-read aligner for Illumina data</td>
</tr>
<tr>
<td>SOAP</td>
<td>Efficient Smith–Waterman aligner for Illumina data</td>
</tr>
<tr>
<td>SMURF</td>
<td>A sensitive aligner for Illumina data that uses the Needleman–Wunsch algorithm</td>
</tr>
<tr>
<td>SOAP2</td>
<td>A faster aligner for determining local relationships within Illumina data</td>
</tr>
<tr>
<td>BWA</td>
<td>Burrows-Wheeler aligner for Illumina and SOLiD data</td>
</tr>
<tr>
<td>BWA-SW</td>
<td>SAM-friendly Smith–Waterman implementation of BWA for long reads</td>
</tr>
<tr>
<td>SHRiMP</td>
<td>Efficient Smith–Waterman mapper with colorspace correction</td>
</tr>
<tr>
<td>FALCON</td>
<td>Fast Alignment of Long reads</td>
</tr>
<tr>
<td>LAST</td>
<td>FAST aligner for Illumina and SOLiD data</td>
</tr>
<tr>
<td>SOAP</td>
<td>SAM-friendly sequence search and alignment by hashing program</td>
</tr>
<tr>
<td>SOAP2</td>
<td>SAM-friendly version of SOAP</td>
</tr>
<tr>
<td>SOAP3</td>
<td>Long-read aligner for Illumina data</td>
</tr>
<tr>
<td>MAQ</td>
<td>A widely used mapping tool for Illumina and SOLiD; now deprecated by BWA</td>
</tr>
</tbody>
</table>

Short read alignment

- Given a reference and a set of reads, report at least one “good” local alignment for each read if one exists
- Approximate answer to question: where in genome did read originate?
- What is “good”? For now, we concentrate on:
  - Fewer mismatches = better
  - Failing to align a low-quality base is better than failing to align a high-quality base

Post alignment: what do you get?

- Alignment of reads including read pairs
- SAM file
- Simplified pileup output
- Read Pair
- CIGAR field

Workflow

Raw Data Analysis
Image processing and base calling

Whole Genome Mapping
Alignment to reference genome

Variant Calling
Detection of genetic variation (SNPs, Indels, insertions)

Annotation
Linking variants to biological information
Discovering Genetic Variation

SNPs

ATCCTGATTCGGTGAACGTTATCGACGATCCGATCGA

CGGTGAACGTTATCGACGATCCGATCGAACTGTCAGC

GGTGAACGTTATCGACGTTCCGATCGAACTGTCAGCG

TGAACGTTATCGACGTTCCGATCGAACTGTCAGCGGC

TGAACGTTATCGACGTTCCGATCGAACTGTCAGCGGC

TGAACGTTATCGACGTTCCGATCGAACTGTCAGCGGC

GTTATCGACGATCCGATCGAACTGTCAGCGGCAAGCT

TTATCGACGATCCGATCGAACTGTCAGCGGCAAGCT

TCGACGATCCGATCGAACTGTCAGCGGCAAGCTGAT

ATCCGATCGAACTGTCAGCGGCAAGCTGATCGATCGA

GATCGAACTGTCAGCGGCAAGCTGATCGATCGATGCTA

TGTCAGCGGCAAGCTGATCGATCGATCGATGCTAG

INDELs

Workflow

Raw Data Analysis
Image processing and base calling

Whole Genome Mapping
Alignment to reference genome

Variant Calling
Detection of genetic variation (SNPs, Indels, insertions)

Annotation
Linking variants to biological information

Where to go to annotate genomic data, determine clinical relevance?

- Human genome mutation database (http://www.hgvs.org/dblist/glsdb.html)
- PharmGKB (http://www.pharmgkb.org)
- Scientific literature

Case-control study design = variable results

| Table 1: Predictions for Disease Relative Risks for Five Individuals |
|------------------|----------|----------|----------|----------|----------|
| Disease          | Natural  | Female 2 | Female 3 | Male 1   | Male 2   |
| Breast cancer    | 1        | 1        |           | 1        |           |
| Colon cancer     |           | 1        | 1        | 1        | 1        |
| Ovarian cancer   |           | 1        | 1        | 1        | 1        |
| Head and neck    |           | -1       | -1       | -1       | -1       |
| Head and neck    |           | -1       | -1       | -1       | -1       |

- Need for Clinical Grade Database
  - Ease of use
  - Continuously updated
  - Clinically relevant SNPs/variants
Cancer Treatment: NGS of Tumor

Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors

Case History

- 78 year old male
- Poorly differentiated papillary adenocarcinoma of tongue
- Metastatic to lymph nodes
- Failed chemotherapy
- Decision to use next-generation sequencing methods

Workflow

- Raw Data Analysis
- Whole Genome Mapping
- Variant Calling
- Annotation

Methods and Results

- Analysis
  - Whole genome
  - Transcriptome

- Findings
  - Upregulation of RET oncogene
  - Downregulation of PTEN

Transcriptome and Whole-exome

- Transcriptome
  - Convert RNA to cDNA
  - Perform sequencing
  - Only expressed genes
  - Can get expression levels

- Whole-exome
  - Use selection procedure to enrich exons
  - No intron data
  - Results depends on selection procedure

A few words about samples...

- Can use formalin-fixed paraffin-embedded tissue for whole-exome or transcriptome sequencing
- Need frozen tissue for whole-genome sequencing
  - Better quality DNA
- Small quantity of DNA needed
  - For whole-exome sequencing, amount off a few slides
Summary

• Gene chips
  – SNPs
  – Expression profiling
  – Copy number variation

• Major steps in NGS
  – Base calling
  – Alignment
  – Variant calling
  – Annotation

• Technology will change but just another test
  – Accuracy
  – Precision
  – Need to validate findings with traditional methods

**Lecture III:** Interpreting genomic information for clinical care

Richard L. Haspel, MD, PhD
Karen L. Kaul, MD, PhD
Henry M. Rinder, MD, PhD

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**Why Pathologists? We have access, we know testing**

- Physician sends sample to Pathology (blood/tissue)
- Just another laboratory test

**What we could test for? Same Stuff**

- Somatic analysis
  - Tumor genomics
  - Diagnosis/Prognosis
  - Response to treatment
  - May change/evolve/require repeat testing
- Laboratory testing
  - Microbiology
  - Pre-natal testing

**What we could test for? Something New**

- Risk prediction
  - Pathologists involved in preventive medicine
    - Predict risk of disease
    - Predict drug response (pharmacogenomics)
- Germline
  - Heritable genomic targets
  - Does not change during lifetime

**What we will cover today:**

- Review current and future molecular testing:
  - Somatic analysis/Prognosis
    - Cancer
  - Laboratory testing
    - Microbiology
    - Pre-natal testing
  - Risk Assessment
    - Pathologists involved in preventive medicine
Diagnosis/Prognosis Timeline: Cancer

- Single gene
  - HER2
- Multi-gene assays
  - Breast cancer

Gene chips/Next generation sequencing of tumors
- Expression profiling
- Exome
- Transcriptome
- Whole genome

Multi-gene assays

<table>
<thead>
<tr>
<th>Gene</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>0.47</td>
</tr>
<tr>
<td>ER Group</td>
<td>-0.34</td>
</tr>
<tr>
<td>PR Group</td>
<td>1.01</td>
</tr>
<tr>
<td>CD88</td>
<td>0.10</td>
</tr>
<tr>
<td>GSTM1</td>
<td>-0.06</td>
</tr>
<tr>
<td>BAX</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

For use in ER+, node negative cancer

Multi-gene assays in breast cancer

- Oncotype similar predictive value to combined four immunohistochemical stains (ER, PR, HER2, Ki-67)
- May offer standardization lacking in IHC
- Need to validate
  - Prospective trials


- Analyzed 8,101 genes on chip microarrays
- Reference= pooled cell lines
- Breast cancer subgroups

Prognostic Value of a Combined Estrogen Receptor, Progesterone Receptor, Ki-67, and Human Epidermal Growth Factor Receptor 2 Immunohistochemical Score and Comparison With the Genomic Health Recurrence Score in Early Breast Cancer

Just another laboratory test


Cancer Treatment: NGS in AML

- Analyzed 8,101 genes on chip microarrays
- Reference= pooled cell lines
- Breast cancer subgroups

Use of Whole-Genome Sequencing to Diagnose a Cryptic Fusion Oncogene

Chen X, et al. JAMA. 2011;305: 1577
Case History

- 39 year old female with APML by morphology
- Cytogenetics and RT-PCR unable to detect PML-RAR fusion
- Clinical question: Treat with ATRA versus allogeneic stem cell transplant

The Findings: Led to appropriate treatment

- Analysis
  - Paired-end NGS
- Findings
  - Cytogenetically cryptic event: novel fusion
- Analysis took 7 weeks
- ATRA Treatment
- Patient still alive 15 months later

Cancer Treatment: NGS of Tumor

Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors

Methods and Results

- Analysis
  - Whole genome
  - Transcriptome
- Findings
  - Upregulation of RET oncogene
  - Downregulation of PTEN
Why Pathologists? We have access, we know testing

Would like to identify tumor, know prognosis, treatment options

Pathologists

Personalized Tumor Treatment Plan

Access to tumor genome

Why pathologists?

“However, to fully use this potentially transformative technology to make informed clinical decisions, standards will have to be developed that allow for CLIA-CAP certification of whole-genome sequencing and for direct reporting of relevant results to treating physicians.”

Welch JS, et al. JAMA, 2011;305:1577

What we will cover today:

• Review current and future molecular testing:
  – Somatic analysis/Diagnosis/Prognosis
  • Cancer
  – Laboratory testing
  • Microbiology
  • Prenatal testing
  – Risk Assessment
  • Pathologists involved in preventive medicine

Laboratory Testing: Micro

• Identifying outbreak source
  – Serotyping
  – Pulsed field electrophoresis
  – Next-generation sequencing analysis

Laboratory testing: Prenatal

• Amniocentesis/Chorionic villus sampling
  – Karyotyping
  – Single gene testing

• Multigene assays
  – “Universal Genetic Test” available for 100+ diseases

• Next generation methods
  – Fetal DNA in maternal plasma, detection of Trisomy 21

Fan HC, et al. PNAS. 2009;106:16988
What we will cover today:

- Review current and future molecular testing:
  - Somatic analysis/ Diagnosis/Prognosis
  - Cancer
  - Laboratory testing
  - Microbiology
  - Pre-natal testing
- Risk Assessment
  - Pathologists involved in preventive medicine

Risk Prediction: Timeline

- Single gene
- Multigene assays
  - Direct-to-consumer
- Next generation sequencing

Hereditary Risk Prediction: How is risk calculated?

- Analysis of SNPs (up to a million)
  - Genome wide association studies (GWAS)
- Case-control studies
  - Odds ratios
- Using odds ratios to determine individual patient risk

Just another test: Case-control study

- Adequate selection criteria for cases/controls
- # of patients = reasonable ORs (<=1.3)
- Assays appropriate
  - Enough variation
  - Proper controls
- Statistics appropriate
- Detect known variants
- Reproducible results
  - Different populations
  - Different samples
- Pathophysiologic basis

Just another test: Selection

- Lung cancer risk
- "Old School Study"
  - Cases and controls were matched based on age, smoking status, race and month of blood collection
- "Genomic Study"
  - Cases and controls were frequency matched by sex, age center, referral (or of residence) area and period of recruitment
Statistics: Classic case-control study

Lung Cancer

+ -

A B

C D

Vitamin E Low Level

AD/BC = Odds ratio (OR) – Relative risk (RR)

GWAS: (Case-control)^N

Lung Cancer

+ -

A B

C D

SNP 1

GWAS: (Case-control)^N

Lung Cancer

+ -

A B

C D

SNP 2

GWAS: (Case-control)^N

Lung Cancer

+ -

A B

C D

SNP 3

GWAS: (Case-control)^N

Lung Cancer

+ -

A B

C D

SNP X

A word about statistics…

- 20 tests, "significant" if p<0.05
- (.95)^20 = chance all tests "not significant"
- 1 - (.95)^20 = chance one test "significant"
- 1 - (.95)^20 = 64%
- Bonferroni correction p = 0.0025
- Need to adjust for number of tests run
- For 1 million SNP GWAS p< 0.00000005

Just another laboratory test

Lagakos SW. NEJM 2006;354:16
Why Pathologists? We have access, we know testing

Pathologists

Personal Risk Prediction

Would like to determine patient risk for disease

Access to patient’s chip results

Not so simple!!

Risk Prediction: Not easy to do!!

- Based on case-control study design = variable results
- No quality control of associations
  - Need for Clinical Grade Database
    - Ease of use
    - Continually updated
    - Clinically relevant SNPs/variations
- Pre-test probability assessment

DTC: A simplistic calculation

Calculating pre-test probability is not so simple

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How about family history? Environment?
Just another laboratory test

Pharmacogenomics may guide care

Need validation in clinical trials

Clinical Risk determination (prevalence X post test probability = clinical risk)

Why Pathologists? We have access, we know testing

Access to patient’s whole genome!

Not so simple!!

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Hereditary Risk Prediction: NGS

Clinical assessment incorporating a personal genome

- 40 year old male with family history of CAD and sudden cardiac death
- Whole genome sequencing performed on DNA from whole blood
- How to approach analysis?

Pharmacogenomics may guide care
- Need validation in clinical trials

Other variants detected

Clinical Risk determination (prevalence X post test probability = clinical risk)

Why Pathologists? We have access, we know testing
- Access to patient’s whole genome!
- Not so simple!!

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Overview: Your estimated lifetime risk

- "Avg" (average risk for your ethnic group = pre-test probability):
- OR from SNP is 0.75
- "You" (post-test probability): 8% x 0.75 = 6%
- Absolute decreased risk: 2%
- "Avg" OR if 80% vs. 60%
- Absolute decreased risk: 20%

- Pharmacogenomics may guide care
- Need validation in clinical trials

Other variants detected
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<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre-test Probability</th>
<th>Post-test Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-lipoic acid</td>
<td>10.0%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Melatonin</td>
<td>5.0%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Arsenic</td>
<td>5.4%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5.5%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.6%</td>
<td>2.4%</td>
</tr>
<tr>
<td>Cancer</td>
<td>5.8%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5.9%</td>
<td>3.5%</td>
</tr>
<tr>
<td>Obesity</td>
<td>5.9%</td>
<td>3.6%</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>5.9%</td>
<td>3.6%</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>6.5%</td>
<td>5.3%</td>
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In the end: Is the info actionable?

- “No methods exist for statistical integration of such conditionally dependent risks”
- Strength of association based on # of Medline articles

Summary

- Genomic-era technologies involve
  - Typical roles of pathologists
  - Cancer diagnosis/prognosis/guide treatment
  - Laboratory testing (e.g., microbiology)
  - New roles for pathologists
  - Predict disease risk
  - Predict drug response
  - We control the specimens

- Just another test
  - Issues with case-control studies
  - Issues of pre- and post-test probability
  - Accurately assessing pre-test probability
  - Need to validate

NEJM. 1994;330:1029
Lecture IV: Genomic Medicine: Communicating with the Patient

Julianne O'Daniel, MS, CGC
Joan Scott, MS, CGC
Elizabeth Varga, MS, CGC
VO Speights, DO
Erynn Gordon, MS, CGC

What will be the pathology report of the future?

Why Pathologists? We have access, we know testing

Physician sends sample to Pathology (blood/tissue)
Access to patient’s genome

Pathologists
Genetic Counselors
Medical Geneticists

Workflow must include communication

Multidisciplinary Teams Needed

• Modern medical technology and practice requires a multidisciplinary approach to medical practice.
• Greater interaction needed between Pathologists, Genetic Counselors and Medical Geneticists

What we will cover today:

• Geneticist training and professional roles
• Current and future of reporting and communicating genetic test results
  – Case 1
    • Single gene tests
    • Direct-to-consumer (DTC) gene chip testing
  – Case 2
    • Whole genome sequencing
The approach

<table>
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<tr>
<th>Ethical and Legal Issues</th>
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<tr>
<td>Pre-test Counseling</td>
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<tr>
<td>Informed Consent</td>
</tr>
<tr>
<td>Testing</td>
</tr>
<tr>
<td>Post-test Counseling</td>
</tr>
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Medical Geneticists

- MD or DO
  - Separate residency training or in combination with other medical specialty
- MD, DO or PhD
  - Biochemical Genetics
  - Molecular Genetics
  - Cytogenetics

Genetic Counselors

- “Genetic counseling is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease.”
- Masters-prepared
  - Medical genetics
  - Risk assessment
  - Communication
  - Psychosocial assessment
- Board certification
- Licensed in a growing number of states

Genetic Counselors

- Many work settings:
- Scope of practice statement:
  - To provide expertise in clinical genetics
  - To counsel and communicate with patients on matters of clinical genetics;
  - To provide genetic counseling services in accordance with professional ethics and values.
- Clinical expertise spans:
  - Prenatal, pediatric, cancer, adult-onset conditions

Geneticists as a Resource

- Bridge gap between healthcare providers, pathologists, and patients
- Develop educational material
- Consult about ethical issues
- Develop provider and patient-friendly reports

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Case 1 – Single Gene Testing

- A 38 year old woman has a strong family history of breast cancer.
- Affected family members not available for testing
- She is referred for genetic counseling

Guidelines exist for BRCA testing

The USPSTF recommends that women whose family history is associated with an increased risk for deleterious mutations in BRCA1 or BRCA2 genes be referred for genetic counseling and evaluation for BRCA testing. Rating: B Recommendation.

Genetic Counseling for Breast Cancer

- ~ 1 in 8 US women will develop breast cancer
  - Not uncommon to have >1 sporadic case in a family
  - Only 1 in 300 to 400 have a known mutation
  - Several steps in the process of genetic counseling

Pre-test counseling is crucial

- Gathering information
  - Medical, Family and Social histories
- Risk assessment includes:
  - Family Hx/Family member testing
  - Medical Hx
  - Risk scoring programs (BRCAPRO, Gail)

Pre-test counseling in crucial

- Presenting information
  - Suspected differential and/or testing strategy
  - Testing process, options
  - Facilitate decision-making
  - Patient/family concerns and expectations about genetics and testing
Implications of Results: Context Matters

- The informational and counseling needs depend on context and indications for testing
  - Examples:
    - Establish or confirm a diagnosis (Gaucher Disease)
    - Reproductive decision-making, carrier testing, fetal testing (cystic fibrosis)
    - Predict risk for future disease (BRCA, Huntington's Disease)
    - Aid management decisions (e.g., tumor sequencing)
- Ethical issues vary by context

Ethics: Use of Genetic Info

- Genetic Information Nondiscrimination Act (GINA)
  - Protects against use of genetic information in health and employment
  - Does not protect in disability or life insurance or symptomatic disease
  - Active duty military and federal employees are currently exempt from GINA protections
  - Has not been tested in the courts

Ethics: Prenatal and Pediatric Testing

- Pre-implantation genetic diagnosis (PGD)
  - Identify unaffected embryos for implantation
  - What types of genetic disease may be included?
- Testing of fetuses or minors for adult-onset disorders
  - Generally NOT recommended in the absence of increased risk for pediatric symptoms or treatment options.
  - Even carrier testing may have consequences.

The approach

- Pre-test Counseling
- Informed Consent
- Testing
- Post-test counseling

Informed Consent May be Required

- Regulations for genetic tests vary by state
- May be institutional requirement for review by Risk Management
- Consent forms include practical issues:
  - What and why testing is being done
  - Risk, benefits, and limitations of testing
  - How results will be conveyed
  - Potential results and implications
    - Effect on relatives
    - Cost and insurance coverage
    - Privacy and confidentiality
The approach

Pre-test Counseling
Informed Consent
Testing
Post-test counseling

Ethical and Legal Issues

What Geneticists Need to Know about a Test

• Is the lab reliable?
• Coordinate testing
  – Sample collection
  – Communications with lab
• What are the limitations in terms of answering the clinical question?
  – Detection rate
  – Method/Coverage of target
• Potential need for follow-up testing

Possible test results

• Positive (known pathogenic mutation)
• Negative
  – Absence of known pathogenic mutation—Need to discuss residual disease risk
  – Absence of known familial pathogenic mutation—Need to discuss population risks and screening
• Unknown Significance
  – There is no or limited evidence about the pathogenicity of the identified variant(s)
  – Additional testing and/or future re-interpretation is necessary

Post-test Counseling

• Review reasons for testing
• Review test result(s)
• Assess comprehension and coping
• Discuss plan for additional evaluation and/or treatment
• Follow-up

Implications of Results: Context Matters

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Case 1– Single Gene Testing

• Patient tests positive for BRCA
  – S1970X (6137C>A) mutation in BRCA2.
  (Not an Ashkenazi Jewish mutation)
  – Implications for patient
• Ethical issue
  – Other family members?
Dear [ ],

I recently had genetic testing to help me understand my risk of developing cancer. I was tested for inherited changes (or mutations) in the *BRCA1* and *BRCA2* genes. My test identified a mutation that runs in our family (relatives related by blood).

This test result means that I have Hereditary Breast and Ovarian Cancer syndrome. Fortunately, there are medical options to reduce my risks, which is why knowing about this mutation will be very helpful.

It is possible that someone in our family besides me may have a *BRCA1* or *BRCA2* mutation. I am writing to all of the relatives who may be at risk to also have this mutation. You may want to talk to your doctor about whether genetic testing makes sense for you.

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**Case 1– Single Gene Testing**

The patient’s 35 year old cousin is interested in learning more about her breast cancer risk but decides to pursue testing on her own through a DTC company.

<table>
<thead>
<tr>
<th>carrier status</th>
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<tbody>
<tr>
<td>BRCA1/Mutation</td>
</tr>
<tr>
<td>BRCA2/Mutation</td>
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- Does not include all mutations such as S1970X (6137C>A) mutation in *BRCA2*.

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**DTC: A simplistic calculation**

How about family history? Environment?

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**Risk Prediction: Not easy to do!!**

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- No quality control of associations
  - Need for Clinical Grade Database
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**DTC Genetic Tests: Positive Aspects?**

- Educate consumers
- Provide direct access
- Empower consumers to take charge of their health
- Motivate behavior change to improve health outcomes
- Provide confidential testing

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DTC Genetic Tests: Points to Consider

- Questionable benefits
- Qualification of the lab
- Clinical validity of the test(s)
- Claims made about the test(s)
- Privacy
- Clinical application
  - Appropriateness of tests ordered
  - Interpretation of results
  - False reassurance if “normal” or unwarranted concern if “positive”
  - Forgoing standard treatments
  - Seeking unnecessary treatments

Effect of Direct-to-Consumer Genomewide Profiling to Assess Disease Risk
Cinnamon I. Bloss, Ph.D., Nicholas J. Schreiber, Ph.D., and Eric J. Topol, M.D.

Sample swaps at 23 and Me: a cautionary tale
By Daniel MacArthur | June 7, 2010 | 6:00 a.m.

- No evidence for anxiety
- No evidence for change

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Case 2 – Risk Prediction with NGS

- Patient assessed by cardiologist and genetic counselor due to family history of vascular disease and sudden death
  - 40 yo, no significant past medical history
    - Exercises regularly, no medications
    - Normal clinical exam
    - Normal lipid panel, electrocardiogram, echocardiogram and cardiopulmonary exercise test
    - Social history: highly educated male, professor of bioengineering

Pharmacogenomics may guide care

- 64 clinically relevant pharmacogenomic variants, 12 novel, non-conservative amino acid changing SNPs
- Most relevant to current care:
  - CYP2C19 variant: clopidogrel
- Most relevant to future care
  - Variants affecting warfarin dosing, response to statins
Rare Cardiac Variants – Possibly Pathogenic

- MYBPC3 Arg326Gln - originally associated with late onset hypertrophic cardiomyopathy
  - Later found in multiple controls
  - Conclusion: probably benign
- TMEM42 Met41Val - in 1 of 150 probands with ARVD/C
  - Test other family members to help assess relevance?
- DSP Arg1838His - entirely new variant

Unexpected Findings

- 3 variants in 2 genes associated with hemochromatosis
  - No family history
  - No current clinical evidence of disease in patient
  - How should management be changed? Serum ferritin monitoring?
- Gene variant implicated in parathyroid pathology
  - Osteoarthritis in family and knee pain in patient related to this?

Clinical Risk determination (prevalence X post test probability = clinical risk)

Pre-test Probability: Population vs Individual

- No methods exist for statistical integration of such conditionally dependent risks
- Strength of association based on # of Medline articles

To the Nth power for genomics
Counseling: Genomic Testing

- Same principles
  - Setting appropriate expectations
  - WGS is limited by our current biological understanding
- Communicating multiple complex results effectively
  - Prioritization of most significant results
  - Timing of communication (how much at once?)
  - Method of communication (written, verbal, electronic)
  - Incidental findings
  - Familial implications of results
  - Need for ongoing communication as interpretation of results evolves

Ethics: To the Nth Power

- Informational overload and patient comprehension
- Patient autonomy to learn/refuse results
- What information should be returned?
- Lab and clinical liabilities if results are not disclosed or genomic regions analyzed?
- How to store results in an accessible but private manner?
- What information to return when testing minors?

Genomics Requires New Clinical Models

- Lengthy, multiple pre-post-test sessions
- Reimbursement for broad-based testing
- Data reporting and storage in medical record systems
- Informed consent
  - “The patient received education and counseling before signing the consent form and throughout testing and follow-up.”

Summary: To the Nth Power

- Core principles apply
- Huge amount of data
- Difficulties in interpretation
- Reimbursement
- Multiple ethical issues
  - Informed consent

Multidisciplinary Teams Needed

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<td>Integrated data interpretation</td>
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