Disclosures

• Medical Director, UAB Medical Genomics Laboratory
• Chair, Medical Affairs Committee, Children’s Tumor Foundation
• Grant Funding: NIH, Department of Defense, Novartis
• Advisory Boards: Novartis NF Advisory Board, March of Dimes
Newborn Screening

Shortly after birth, blood is taken from Laura’s heel and sent to the State Newborn Screening Laboratory. Her parents are told that this is a routine test. No problems are found, and no follow-up is needed.

http://www.sigmaxi.org/amsci/articles/02articles/millingoncap3.html
"We found an unexpectedly high proportion of literature-annotated disease mutations that were incorrect, incomplete, or common polymorphisms."

Immobilization/Amplification

DNA Synthesis/Detection


Diagnostic Testing

Laura is now 3 and her brother Seth is 5. Seth has been experiencing developmental problems, and is diagnosed as having autism.
Genetic Evaluation in Autism

Array CGH
UAB Cytogenetics Laboratory
The Diagnostic Odyssey

Genomic Diagnosis
Genome Annotation

Secondary Findings

Preconceptional Testing

Laura is now married. She and her husband are considering starting a family and meet with her obstetrician-gynecologist. They are both of Northern European ancestry and are offered carrier testing for cystic fibrosis.
CF Carrier Screening

Inquire:
- Mucolipidosis IV
- Niemann-Pick A
- Fanconi anemia
- Bloom syndrome
- Gaucher disease

Ashkenazi Jewish
- Tay-Sachs disease
- Cystic fibrosis
- Canavan disease
- Familial dysautonomia

African/Asian/Mediterranean
- Hemoglobinopathies

Panethnic
- Cystic fibrosis
- Spinal muscular atrophy
Laura and her Tom are indeed found to both be cystic fibrosis carriers. They elect to have prenatal diagnosis by amniocentesis at 16 weeks of pregnancy. The fetus is found to be a CF carrier.
Prenatal Diagnosis

- amniocentesis
- chorionic villus biopsy
- preimplantation diagnosis

Next Generation Prenatal Screening

Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing

Diana W. Bianchi, MD, Laurence D. Platt, MD, James D. Goldberg, Amy J. Scherzer, MD, and Richard P. Rava, MD, on behalf of the Ma Accurately diagnose fetal aneuploidy (MELISSA) Study Group

DOI: 10.1016/j.ajog.2013.05.045
Laura is now 45. She has just learned that her older sister Abby, age 49, has been diagnosed as having breast cancer. She is concerned about her own risks, given that there is a family history of others with breast cancer.
Breast Cancer Prevention

The New England Journal of Medicine

Cumulative Proportion without Breast or Ovarian Cancer

Survival to 72 Months

BRCA1+ BRCA1-
The Redactome

Laura is now 60 years old. She has been in good health. She and her husband have heard about the possibility of having genomic testing, and explore the possibilities on the internet.
Genome-Wide Association Studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin</td>
<td>IL-4, IL-13</td>
</tr>
<tr>
<td>Cluster of differentiation</td>
<td>CD14</td>
</tr>
<tr>
<td>β2-Adrenergic receptor</td>
<td>B2AR</td>
</tr>
<tr>
<td>Human leukocyte antigen</td>
<td>HLA-DRβ1, HLA-DQ81</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>TNF</td>
</tr>
<tr>
<td>High-affinity IgE receptor β</td>
<td>FCER1B</td>
</tr>
<tr>
<td>Interleukin-4 receptor</td>
<td>IL4RA</td>
</tr>
<tr>
<td>Disintegrin and metalloproteinase domain 3</td>
<td>ADAM33</td>
</tr>
</tbody>
</table>

Bierbaum, S., Heinzmann, A. Resp Med

doi:10.1016/j.rmed.2007.01.018
The “Dark Matter” in the Genome


Direct to Consumer Genomic Testing
Pharmacogenetics

**Your Genetic Data**

<table>
<thead>
<tr>
<th>Name</th>
<th>Confidence</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin (Coumadin®) Sensitivity</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Increased</td>
</tr>
<tr>
<td>Alcohol Hypersensitivity</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Typical</td>
</tr>
<tr>
<td>Alcohol Consumption, Braving and Risk of Hepatocellular Carcinoma</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Typical</td>
</tr>
<tr>
<td>Cephaloglycin (Plavix®) Efficacy</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Typical</td>
</tr>
<tr>
<td>Fluoroquinolone Toxicity</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Typical</td>
</tr>
<tr>
<td>Response to Hepatitis C Treatment</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Typical</td>
</tr>
<tr>
<td>Pseudocholinesterase Deficiency</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Typical</td>
</tr>
<tr>
<td>Oral Contraceptives, Hormone Replacement Therapy and Risk of Various Thrombosis</td>
<td>❝ ❝ ❝ ❝ ❝</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>Caffeine Metabolism</td>
<td>❝ ❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Slow Metabolizer</td>
</tr>
<tr>
<td>Hepatitis C Treatment Side Effects</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Slow Metabolizer</td>
</tr>
<tr>
<td>Metformin Response</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Higher Odds of Positive Response</td>
</tr>
<tr>
<td>Antidepressant Response</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>See Report</td>
</tr>
<tr>
<td>Beta-Blocker Response</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>See Report</td>
</tr>
<tr>
<td>Fluoxetine Toxicity</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Typical Odds</td>
</tr>
<tr>
<td>Heroin Addiction</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Typical Odds</td>
</tr>
<tr>
<td>Luminosin (Pregabalin®) Side Effects</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Typical Odds</td>
</tr>
<tr>
<td>Natalizumab Treatment Response</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>See Report</td>
</tr>
<tr>
<td>Postoperative Nausea and Vomiting (PONV)</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Higher Odds</td>
</tr>
<tr>
<td>Response to Insulin Basal Therapy</td>
<td>❝ ❝ ❝ ❝ ❝ ❝ ❝</td>
<td>Increased Odds of Responding</td>
</tr>
<tr>
<td>Statin Response</td>
<td>❝ ❝ ❝ ❝ ❝ ❝</td>
<td>See Report</td>
</tr>
</tbody>
</table>

The genotyping services of 23andMe are performed in LabCorp's CLIA-certified laboratory. The tests have not been cleared or approved by the FDA.
Genomics and Epigenomics

Environmental exposures modify gene expression

ANTENATAL EFFECTS
At birth: emerging differences in immune

POSTNATAL EFFECTS
Evolving phenotype / Trans-generational effects?

Genetics
Inherited genotype
Complex interactions
Viruses
Bacteria
n-3PUFA
Antioxidants
Prebiotics
Folates
Allergens
Pollutants

Personalized Genomics

Where Will Personal Genomes Live?

Personalized

UAB Medicine
NIH Undiagnosed Disease Program

Strategic Initiative Genomics & Proteomics

The principal strategic goal in genomics and proteomics at UAB is to empower investigators, which means to provide support to experienced investigators and to help “bootstrap” less experienced investigators to build genomics and proteomics into their research.

Heflin Center for Genomic Sciences

Director
Bruce Korf

Genomics Core
Molly Bray
Proteomics
Jim Mobley
Analytic & Epidemiologic Genomics
Hemant Tiwari
Personalized Medicine
Bruce Korf

Sequencing
Mike Crowley
Bioinformatics
David Crossman
Genomics Pipeline at UAB

HEFLIN CENTER FOR GENOMIC SCIENCES
Low to High Throughput Genomic and Genetic Assays
Focused Next Gen Sequencing and GX

HUDSON ALPHA INSTITUTE
Large Scale Next Gen and Specialized Sequencing Projects,
High Volume Genomics Assays

Study Design and Consultation
Sample Acquisition
- Clinics
- Hospital
- Offsite
Sample Processing
- Logging and Storage
- DNA/RNA extraction
- Assay Preparation
Whole Genome and Custom
- Sequencing
- Gene expression
- Genotyping
- Methylation
Data Analysis
- Sequence analysis
- Gene expression analysis
- Genotyping analysis
- Methylation analysis

University of Alabama at Birmingham
Heflin Center for Genomic Science
Next Generation Sequencing Technology and Services at UAB

- Illumina Sequencing Resources (in collaboration with the Stem Cell Institute):
  - Hi-Seq 2000 and GAIIx Next Generation Sequencers
  - cBot and Covaris S2 for Illumina HiSeq2000 and GAIIx Next-Gen library prep

Next Generation Sequencing at UAB

- HiSeq2000
  - The HiSeq2000 can produce ~600 billion bases (Gb) of sequence per run (300 billion per flowcell with 2 flowcells)
  - The GAIIx can produce up to 95 billion bases (Gb) of sequence per run

- NGS Assays include:
  - mRNA-Seq
  - ChIP-Seq
  - Exome Sequencing
  - Whole Genome Sequencing
  - Targeted Re-sequencing
  - Microbiome
UAB Illumina Technology and Services

- Illumina Array Resources
  - iScan - High density and high throughput genotyping, expression and methylation arrays
  - BeadXpress - Medium density and throughput genotyping and methylation assays
  - Tecan robotics for accurate and efficient liquid/sample handling

Illumina Microarrays Offered

<table>
<thead>
<tr>
<th>Chip</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>HumanOmini5.0-4</td>
<td>&gt;4.3 million</td>
</tr>
<tr>
<td>HumanOmini-2.5-8 + 2.5S</td>
<td>&gt;2.5 million</td>
</tr>
<tr>
<td>HumanOmini-1 Quad + 1S</td>
<td>&gt;1,000,000 (new design)</td>
</tr>
<tr>
<td>HumanOmini Express-12</td>
<td>700K + up to 200K custom</td>
</tr>
<tr>
<td>HumanExome + OmniExpressExome</td>
<td>&gt;250K</td>
</tr>
<tr>
<td>Human Linkage-24</td>
<td>~6100</td>
</tr>
<tr>
<td>Human CytoSNP</td>
<td>~300K</td>
</tr>
<tr>
<td>Select custom arrays</td>
<td>Custom up to 1 million</td>
</tr>
<tr>
<td>High density genotyping arrays for cow, dog, maize, pig, and sheep</td>
<td>~50K-700K</td>
</tr>
<tr>
<td>Human HT-12 gene expression BeadChip</td>
<td>~47 K curated and non-curated transcripts and ESTs</td>
</tr>
<tr>
<td>Mouse WG6 gene expression BeadChip</td>
<td>~45 K curated and non-curated transcripts and ESTs</td>
</tr>
<tr>
<td>Mouse Ref-8 gene expression BeadChip</td>
<td>~26 K curated transcripts and ESTs</td>
</tr>
<tr>
<td>Human Methylation450</td>
<td>~450K putative methylation sites</td>
</tr>
</tbody>
</table>
### Microarray vs. NGS: Cost Comparison

<table>
<thead>
<tr>
<th>Assay</th>
<th>Microarray Cost*</th>
<th>Next Generation Sequencing Assay</th>
<th>Cost*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene expression</td>
<td>$245-$650</td>
<td>mRNA-Seq</td>
<td>$620$§</td>
</tr>
<tr>
<td>Methylation</td>
<td>$365</td>
<td>Methyl-Seq</td>
<td>$650-1800$†</td>
</tr>
<tr>
<td>ChIP-Chip</td>
<td>$550</td>
<td>ChIP-Seq</td>
<td>$650-1800$†</td>
</tr>
<tr>
<td>Genotyping</td>
<td>$80-$620</td>
<td>Whole exome</td>
<td>$1,250$§</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole genome</td>
<td>$5,000$ª</td>
</tr>
</tbody>
</table>

*Prices include labor and consumables and are subject to change
§Price reflects running 28 samples per flowcell on HiSeq2000
†Prices reflect running several samples per lane v. one sample per lane
ªPrice is for running 3 genomes per flowcell

NGS analysis is always going to provide more comprehensive data than an off-the-shelf microarray.

### Microarray vs. NGS: Sample Quality and Quantity

<table>
<thead>
<tr>
<th>Assay</th>
<th>Microarray Amount</th>
<th>Next Generation Sequencing Assay</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene expression</td>
<td>~300 ng total RNA</td>
<td>mRNA-Seq</td>
<td>1µg of Total RNA</td>
</tr>
<tr>
<td>Methylation</td>
<td>500 ng DNA</td>
<td>Methyl-Seq</td>
<td>10ng* of ChIP’d DNA</td>
</tr>
<tr>
<td>ChIP arrays</td>
<td>ChIP’d DNA</td>
<td>ChIP-Seq</td>
<td>10ng* of ChIP’d DNA</td>
</tr>
<tr>
<td>Genotyping</td>
<td>200-400 ng DNA</td>
<td>Whole exome</td>
<td>2-3µg DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole genome</td>
<td>1µg DNA</td>
</tr>
</tbody>
</table>

All assays work best with good quality, un-degraded sample.

Illumina has developed “rescue” reagents for FFPE samples that can greatly increase yield.
Data Analysis “Road Map”

- Several groups on campus with capabilities to analyze NGS data:
  - Heflin Genomics Core – David Crossman, PhD
  - Statistical Genetics – Hemant Tiwari, PhD
  - Pathology Group – Jonas Almeida, PhD
  - CCTS – Elliot Lefkowitz, PhD
  - CCC Statistics Core
- Creation of an analysis “clearing house” web page
  - General information on data complexity
  - Mechanism for triaging workflow and balancing work queues
  - Links to sites for analysis

---

UAB Program in Genomics & Proteomics

- Genomics & Proteomics Study Navigator
  - Consultation service
  - Inventory
  - Needs assessment
  - Genomics & proteomics roadmap
  - Business case for master’s level study consultants

- Genomics & Proteomics Educational Coordinator
  - Education & outreach program
  - Innovative educational offerings

Michelle Amaral, PhD
Heflin Center/CCTS
www.heflingenetics.uab.edu

N. Susan Huffman, PhD
HudsonAlpha
genomics@uab.edu
We tend to overestimate the effect of a technology in the short run and underestimate the effect in the long run.

Amara’s Law