Prenatal Genetic Screening and Diagnosis
Information is not Knowledge

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Professor, Department of Obstetrics, Gynecology
University of Florida
Perspectives of the Human Genome Project

Medical genetic analysis to determine disease risk profile

Genome structure and function
- Genomics Heterogeneity
- Gene Products mRNA
- Architectural Complexity Proteome

Molecular archaeology
- Origin of humans
- Molecular archeology

Evolutionary past

Model-Organisms
- Diagnosis and Gene-Therapy
- Pharmacogenetics
- Molecular Pathophysiology

Medical genetics

Computational Tools
- Microarrays
- Transcriptional profiling
- Serial analysis of gene expression
- Haplotype mapping

Access to information
- Privacy/Discrimination
- Validation of genetic tests
- Ethical principles
- Use of genetic technology for "Customization" of individuals or human gene pool?

Public policy

J LaBaer . Genetics in Medicine 2002;4:2s-9s
Evolutionary biology: Concepts

(Based on genetic variation and population genetics including natural selection, founder effect and genetic drift)

Lineage splitting

Specialization

Adaptation

Innovation

Fact of evolution

Novel body part by regulatory interactions in developmental genes
Two distinct initial steps for a morphological innovation

1. An epigenetic side effect or other evolutionary change in the body leading to a novel physical structure in the organism
2. Genetic consolidation and individuation of the novel structure

Wagner GP. J Exp Zool 304B:580; 2005
Relevance of Innovation
Sub-classification of Mammals for Perinatologist
According to use or not of uterus/placenta for reproduction

Mammals = Mammary Glands & Hair

- Monotreme
  (Ornithorhynchus)

- Meta-Therian
  Marsupial

- Eu-Therian
  (Placenta)
Human Genome Sequence

Medical genetic analysis to determine disease risk profile

Variations = Submicroscopic (~1 – 10,000bp Insertions, deletions, duplications, inversions)
Polymorphism = CNV with population frequency >1%

Next-generation sequencing:
Computerized alignment process of parallel reads of thousands of pieces of DNA

- DNA Library 1, Individual 1
- DNA Library 2, Individual 2
- DNA Library 3, Individual 3

Omics” platforms, including Single Nucleotide Polymorphisms (SNPs) genotyping, Next-generation sequencing and array Comparative Genomic Hybridization (aCGH) are allowing for determination of Position of variants and Frequencies
The “news” in Prenatal Screening and Diagnosis

**fFDNA in Maternal Circulation and Fetal CGH chromosomal analysis**

- Direct SRY gene analysis for X-linked conditions
- Direct gene analysis for RhD factor
- Direct analysis of Monogenic disorders inherited from the father
- Aneuploidy 21, 18, 13, X and Y analysis
  1) Htz SNPs (2:1 haplotype ratio in Trisomy Vs 1:1 in Diploidy)
  2) Distinct DNA markers and allele ratio dosage with relative to a reference DNA sequence from other chromosome (3:2 Vs 2:2) or an independent control sample
  3) Ratio dosage of amplified DNA regions
- Translational Sciences in Perinatal Medicine

**Deducing the fetal genome using Maternal blood samples**

Fetal Microarray Comparative Genomic hybridization
Oligonucleotide probes 25-75bp/Targeted 100-200 kb BAC
Hillman et al. UOG, 2012
Structure of the Chromosome

Chromosome Microarray Analysis (CMA) = Electronic FISH study

~11kb deletion on chromosome 8 revealed by CGH
Blue line = individuals with two copies
Red line = individual with zero copies

Standard G-band karyotyping can detect abnormalities at a resolution of 5-10 Mb. CMA have 1 Kb resolution which is smaller than the average gene. It is informative in ~4-10% of patients with abnormal US findings and normal karyotype but also has the challenge of ~1 in 60 detection of variants of unknown clinical significance (VOUS)
Prenatal Chromosomal Microarray

- Currently, allows for high resolution evaluation of constitutional cytogenetic abnormalities with platforms working in any tissue—Fantastic!

- **The limitations of these techniques are on the clinical interpretation of findings:** Absence of HTz (genome identical by descents?), Ploidy?, Balanced rearrangements?, Low level mosaicism?, CNV not tested on the platform?, Point mutations?, Gene expression or methylation with effect on phenotypes?, and CNV of unknown significance on current databases? (~1/50 amnios)

* I use prenatal targeted arrays in cases selected based on: Abnormal Ultrasound findings, Ambiguous karyotype results, Marker chromosomes, F. Hx of known chromosome imbalances or parents with a balanced translocation
Evidence of Fetal-Maternal Micro-chimerism
“bi-Directional Cell traffic”

- Incidence of malignancy in pregnancy 1 in 1000 pregnancies
  - Primary Breast Cancer
  - Melanoma is most common metastatic malignancy
  - Leukemia
- 50 reported cases of maternal malignancies with metastases to the placenta and 14 cases with documented maternal-to-fetal metastases through the placenta

Types of Cells
- Fetal nucleated RBCs
- Lymphocytes: persistence in maternal circulation in postpartum years
- Trophoblasts: rapidly cleared from circulation might be the ideal source

Estimated # of fetal cells in maternal circulation = 1/100,000-10,000,000

Maternal and paternal haplotype inheritance using a “shot gun approach” and PCR-based target selection
Massively Parallel Sequencing (MPS) of DNA fragments in maternal plasma

cfDNA is isolated and sequenced by MPS, generating millions of sequence reads that are then aligned to sites from a reference human genome (tags) and counted for determination of the chromosome ploidy status.

MATERNAL BLOOD SAMPLE

MATERNAL AND FETAL CELL-FREE DNA

CELL-FREE DNA SEQUENCED VIA MASSIVELY PARALLEL SEQUENCING (MPS)

ALIGNMENT AND COUNTING

Chromosome 21
No Aneuploidy

Chromosome 21
Aneuploidy

verifi® prenatal test
Dual Threshold

Single Threshold Method

Detected
Aneuploidy Suspected
Not Detected

Diploid

Trisomy

e-mail: aswanson@verinata.com
Non Invasive Prenatal Testing (NIPT) (fFDNA in Maternal Circulation)

<table>
<thead>
<tr>
<th></th>
<th>Natera’s Panorama</th>
<th>Verinata’s Verifi</th>
<th>Sequenom’s MaterniT21 PLUS</th>
<th>Ariosa’s Harmony</th>
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</thead>
<tbody>
<tr>
<td>Trisomies tested</td>
<td>13, 18, 21</td>
<td>13, 18, 21, sex chromosomes</td>
<td>13, 18, 21, sex chromosomes</td>
<td>13, 18, 21</td>
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<tr>
<td>Monosomy tested</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>Genetic testing method</td>
<td>Single nucleotide polymorphism</td>
<td>Massively parallel sequencing</td>
<td>Massively parallel sequencing</td>
<td>Chromosome-selective sequencing</td>
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<tr>
<td>Sensitivity</td>
<td>92-99%</td>
<td>87-99%</td>
<td>92%-99%</td>
<td>80-99%</td>
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<td>Accuracy</td>
<td>100%</td>
<td>100%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
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<tr>
<td>Earliest gestational age</td>
<td>9 weeks</td>
<td>10 weeks</td>
<td>10 weeks</td>
<td>10 weeks</td>
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<tr>
<td>Price</td>
<td>$1,495</td>
<td>$1,500</td>
<td>$2,762</td>
<td>$795</td>
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Noninvasive Prenatal Genetic Tests Compared

Paternity testing: Natera (Panorama)
Twins: Sequenom (MaterniT21) & Verinata (Verifi)
Rh(D): Sequenom

Allelic-polymorphisms & Phenotype

**e.g. AAGGGAT to AAGGAAT**

Humans cells have 2 sets of Homologous chromosomes (*Diploid*), *thus* 2 copies of each gene (*Allele*). If both alleles have identical nucleotide sequence the individual is *Homozygote*; if the allele sequences are different the individual is *Heterozygote*.

The DNA of any two individuals will show a difference every 1000 nucleotides.

At a population level, allelic variations can be expressed in different proportions among ethnic groups. Those *de-novo nucleotide differences or Indels* that are transmitted throughout generations reaching a frequency of 1% become *Polymorphisms*.

**SNP**: 2 sequenced DNA fragments from different individuals contain a difference in a single nucleotide (*Indel*-Insertion/Deletion of nucleotide).

The degree and pattern of “dominance” (among alleles within a loci) can vary for many phenotypic traits defying simple Mendelian-categorizations. These traits require modeling using Polygenic and Multifactorial - environmental - Interactions.
Traditional assays that use “amplicons” typically rely on a putative single-copy gene reference assay to normalize the DNA input for downstream interpretation in comparison to a reference genome. Single-copy reference assays may no longer be a reliable indicator of DNA input due to new insights into the presence of a complex chromosome composition - in both number and structure -


### Pediatric hospital admissions in North America, 1998

<table>
<thead>
<tr>
<th></th>
<th>Seattle</th>
<th>Montreal</th>
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<tbody>
<tr>
<td><strong>Chromosomal</strong></td>
<td>0.6%</td>
<td>0.4%</td>
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<tr>
<td><strong>Single gene</strong></td>
<td>3.9%</td>
<td>6.9%</td>
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<tr>
<td><strong>Polygenic</strong></td>
<td>48.9%</td>
<td>29.0%</td>
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<tr>
<td><strong>Nongenetic</strong></td>
<td>46.6%</td>
<td>63.7%</td>
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<tr>
<td><strong>No. of admissions</strong></td>
<td>4,115</td>
<td>12,801</td>
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Gelehrter, Collins and Ginsburg
*Principles of Medical Genetics*

**Genetic testing refers to:** the measurement of DNA, RNA, chromosomes or proteins that reflect changes in genes or gene products associated with specific disorders or phenotypes.
Newborn Screening, in FL

Screening has spared thousands from suffering through pre-symptomatic diagnosis and therapy.

- Tandem mass spectrometry to screen for 31 metabolic disorders
- Hearing loss
Why prenatal genetic screening?

• Identify patients that would benefit from genetic counseling
• Reduce health care costs by targeting more expensive diagnostic testing to high risk populations
• Early treatment of affected individuals
• Identify Individuals that might benefit from reproductive testing to bypass the odds of affected child
Look for factors that put a pregnant woman at a greater than the background Risk for having a newborn or child with a serious Medical condition.

Why? In USA, identification of these high risk patients allows for making informed Reproductive decisions.
Reproductive Genetics: When?

• **Pre-conception screening for couples at increased risk based on family history or background:** this may be the most helpful and cost efficient by allowing time for proper information and evaluation of the partner.

• **Pre-implantation screening:** to reduce the odds of affected fetus/abortion and pregnancy loss in infertile or at risk couples?

• **Pre-natal screening:** fetal/partner identification. Consider unknown paternity (1/10), Father unavailable/ reluctant to be tested/cannot afford screening/ health care disparities.

**Genetic counseling:** avoids stigmatization of patients /family members, allows for discussion of alternative testing options with risks and efficiencies as well as follow up when necessary.
Genetic Screening, Testing and Management

Genetic screening

Evaluation of an unaffected person for carrier status of heritable condition (Pedigree. Screening panel utilized?)

Genetic testing / Management

Analysis of human DNA, RNA, chromosomes, protein, or metabolites to predict or not the occurrence of a genetic condition in the newborn with management implications

Knowledge

- **Informed Consent**: after understanding the nature and clinical course of the disorder, the purpose of the test with pros, cons and implications for the patient, family members and society

- **Actions after the genetic Analysis result**: In a moment of crisis couples expecting a child must have appropriate follow up and support
Screening: Family history “Sweeps”

- 7 Questions will elicit information indicative of Reproductive Genetic Consultation
  - Family history of genetic conditions?
  - Family history of MR/Autism
  - Family history of perinatal death/birth defects/infant surgery
  - Family history of recurrent miscarriage, Infertility, ART
  - Familial Cancer Syndrome, history of cancer
  - Consanguinity
  - Exposure to Teratogens
  - Positive reproductive genetics screening test

Genetic Consultation: Patient’s Assessment & Pedigree analysis, pros and cons of the alternative Screening & Diagnosis options—Beneficence. Consent prior to Genetic screening/testing or therapy (Autonomy). Plan for Management and Follow-up.
# ACOG: Recommended Genetic Screening Tests

## Aneuploidy & Congenital anomalies & Perinatal complications

<table>
<thead>
<tr>
<th>Marker Utility Window</th>
<th>Free Beta</th>
<th>Nuchal Transl</th>
<th>PAPP-A</th>
<th>AFP</th>
<th>Unovaj Estriol</th>
<th>Intact hCG</th>
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<tr>
<th>Gestational Age</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>22</th>
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## Genetic Screening Tests

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<tr>
<th>Ethnicity</th>
<th>Conditions</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>Sickle Cell &lt;br&gt; Cystic Fibrosis &lt;br&gt; Beta-Thalassemia</td>
<td>1 in 10 &lt;br&gt; 1 in 65 &lt;br&gt; 1 in 75</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>Gaucherie disease &lt;br&gt; Cystic Fibrosis &lt;br&gt; Tay-Sachs disease &lt;br&gt; Dysautonomia &lt;br&gt; Canavan’s disease</td>
<td>1 in 15 &lt;br&gt; 1 in 26 - 1 in 29 &lt;br&gt; 1 in 30 &lt;br&gt; 1 in 32 &lt;br&gt; 1 in 40</td>
</tr>
<tr>
<td>Asian</td>
<td>Alpha-Thalassemia &lt;br&gt; Beta-Thalassemia</td>
<td>1 in 20 &lt;br&gt; 1 in 50</td>
</tr>
<tr>
<td>European American</td>
<td>Cystic Fibrosis</td>
<td>1 in 25 - 1 in 29</td>
</tr>
<tr>
<td>French Canadian, Cajun</td>
<td>Tay Sachs disease</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Hispanic</td>
<td>Cystic Fibrosis &lt;br&gt; Beta-Thalassemia</td>
<td>1 in 46 &lt;br&gt; 1 in 30 - 1 in 50</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>Beta-Thalassemia &lt;br&gt; Cystic Fibrosis &lt;br&gt; Sickle Cell</td>
<td>1 in 25 &lt;br&gt; 1 in 29 &lt;br&gt; 1 in 40</td>
</tr>
</tbody>
</table>
“First look” for Aneuploidies

- **How is it done?:** NT ultrasound measurement between 11 and 13+6 weeks gestation (CRL) *combined* with maternal serum PAPP-A and free-beta-hCG
- **Detection rate at 5% FPR**
- **Advantages:** Timing of results allows for diagnostic CVS
- **Disadvantages:** False positive rate greater than integrated sequential screening test

Snijders et al. 1998
Insulin-like Growth Factor System

IGF

IGF-I,II

IGFBP 1-6
(4-binds IGF-II)

IGFBP
-Proteases.

IGF-Recep.
Clinical performance of Uterine Artery BVWF during pregnancy


~20% of patients with Increased Mean P.I. or bilateral early diastolic notching at ~22 weeks will have preeclampsia, IUGR or PNM

Espinoza J, et al. Identification of Patients at Risk for Early Onset and/or Severe Preeclampsia With the Use of Uterine Artery Doppler Velocimetry and Placental Growth Factor (PlGF). AJOG 2007; 196(4):326


Kugler L, Santolaya-Forgas et al. 1st trimester Combined Screening with Uterine volume/Uterine Doppler as a measure for early adaptation to pregnancy in Singleton and Twin gestations, AIUM 2013, SGI 2014 submitted
2nd Trimester “Quad screening”

- **How is done?:** Maternal serum AFP, uE3, hCG and Inhibin A between 15 and 22 weeks gestation
- **Detection rate:** 80% for T21 and 60% for T18 (higher in older populations) at a false positive rate of 5% (higher in older populations)

- **Advantages**
  - ONTD screening test is included

- **Disadvantages**
  - Higher false positive than “First Look Plus”
  - Lower detection rate than “First Look” or “First Look-Plus”
  - CVS for prenatal diagnosis not available
SECOND TRIMESTER MARKERS FOR DOWN SYNDROME IN SINGLETON PREGNANCIES
Sequential Integrated Test

• How is done?:  **Step 1)** 11-13+6 w Combined tests.  
  **Step 2)** 15-22 weeks maternal Quad test

• Detection rate: 90% for T21 and 60% for T18 at a **FPR of 2%**

• Advantages: Patients with 1 in ~30 risk for T-21 in the 1st step can be offered a CVS/NIPT (Contingency screening). Presently this is the most comprehensive screening test includes **ONTD** and prediction of **Adverse Perinatal outcome** (Preeclampsia and IUGR)

• Disadvantages: Estimated risks provided at 16w?
Using Co-linearity based-Risk for Counseling
~Risk for CVS 1 in 100 and for Amniocentesis 1 in 300

<table>
<thead>
<tr>
<th>Chromosomal risk</th>
<th>1 in 40</th>
<th>1 in 209</th>
<th>- 5</th>
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<tbody>
<tr>
<td></td>
<td>1 in 120</td>
<td>1 in 217</td>
<td>+2</td>
</tr>
<tr>
<td></td>
<td>1 in 1000</td>
<td>1 in 199</td>
<td>+5</td>
</tr>
</tbody>
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**OPINION:** Joaquin Santolaya-Forgas, MD, PhD.
FACMG. FACOG. ASMFM. AIUM, 2013
Limitations of NIPT & Microarray

Parent of origin (Imprinting): e.g. Triploidy and partial Moles

- **IMPRINTING:** Gene Inactivation in chromosomes X, 7, 11, 15)
- Confined placental mosaicism (2% CVS)
- **Trisomic rescue > Uniparental Disomy:** CF & IUGR: isoDisomy, both maternal 7 + deltaF508
  - Relevant given association of growth issues: IUGR followed by overgrowth; ‘Syndrome X’

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type I Paternal</th>
<th>Type II Maternal</th>
</tr>
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<tbody>
<tr>
<td>NT MoM</td>
<td>2.76</td>
<td>0.88</td>
</tr>
<tr>
<td>FB MoM</td>
<td>8.04</td>
<td>0.18</td>
</tr>
<tr>
<td>PAPP-A MoM</td>
<td>0.75</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Open neural tube defects

- Prevalence 1-2/1000 pregnancies
- Screening: 2\textsuperscript{nd} trimester alpha fetoprotein
- Detection rate: 80-90\% of spina bifida; 95\% of anencephaly
- False positive/negative rate reduced by Ultrasound Imaging
Prenatal Ultrasound Anomalies
3-examples of Genetic Disorders

**Beckwith Wiedemann Syndrome (11p15.5)** suspected due to macrosomia, omphalocele, hyperglosia.

PDx to confirm hypomethylation of DMR 2 (KCNQ1OT) in 50-60%, Hypermethylation DMR1 (H19) in 2-7%, Paternal UPD in 10-20%, Mutation CDKN1C (p57; 40% familial and 5-10% de novo). Maternal Translocation/Inversion in 1% of cases

**Fraser Syndrome:** A.R. disorder presenting with bilateral renal agenesis, laryngeal stenosis/ataresia, cryptophthalmos, syndactyly

**Heart Defects:** 5-10% due to 22q deletion that are familial in ~50% of cases (recurrence risk 50% if inherited Vs 2-3% if de novo). Other genetic abnormalities associated with heart defects are Trisomy 13, 18, 21, 45X, single gene disorders, multifactorial, and teratogenic exposures including maternal diabetes

**NOTE:** Standard Karyotype is informative in ~30% of the genetic disorders with US-abnormalities
Patient’s Responses to Screening Acceptance of Amniocentesis in Patients at High Risk for Aneuploidy

Contribution of the Genetic Counselor and Referral Source
UI-Chicago. Div Reproductive Genetics and Fetal Medicine, 1997

According to Genetic Counselor and Indication

WCP 2001

(*) P<0.05; (+) P=0.28
Acceptance of Amniocentesis in High Risk Patients for Aneuploidy

Santolaya et al. After (+) Prenatal Screening: 433; TTUHSC 2002
Acceptance of Amniocentesis after (+) QUAD Screening test by Maternal age

Texas Tech University. Div Reproductive Genetics, Fetal Medicine and Ultrasound. SGI, 2004
Acceptance of Amniocentesis by Cultural Background

Santolaya et al. At Texas Tech. SGI 2004
<table>
<thead>
<tr>
<th>Genetic Counseling for (+) First Look Only</th>
<th>Maternal age &lt; 35 yo</th>
</tr>
</thead>
<tbody>
<tr>
<td>591 cases</td>
<td>153 cases</td>
</tr>
<tr>
<td><strong>Acceptance for CVS</strong></td>
<td></td>
</tr>
<tr>
<td>Mean Estimated risk for T21</td>
<td>38 (25%)</td>
</tr>
<tr>
<td></td>
<td>1 in 58</td>
</tr>
<tr>
<td><strong>Acceptance for AMNIOCENTESIS</strong></td>
<td></td>
</tr>
<tr>
<td>Mean Estimated risk for T21</td>
<td>62 (40%)</td>
</tr>
<tr>
<td></td>
<td>1 in 108</td>
</tr>
<tr>
<td><strong>Declined testing</strong></td>
<td></td>
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<tr>
<td>Mean Estimated risk for T21</td>
<td>53 (35%)</td>
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<tr>
<td></td>
<td>1 in 124</td>
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</table>

Center for Fetal Medicine and Reproductive Genetics.
Santolaya and Wilkins-Haug, SGI 2008
Reproductive medicine acumen with major social impacts for Women

* Regulation of ovarian function and contraception
* Embryo and egg Cryopreservation
* Reproductive genetics - application of a collection of biotechnologies for gene profiling and understanding cellular functions during all of the woman’s life time periods

The impact of reproductive technology was recognized with the Nobel Prize in Physiology or Medicine 2010 awarded to Robert G. Edwards “for the development of in vitro fertilization”. The birth of Dolly on 1996 as the first ever organism to be cloned from adult cells and insights gained from the Human Genome Project will now advance our understandings pertinent to the reproductive process paving the way to additional Noble prizes
Patients counseled for IVF-PGD at Brigham and Women’s hospital, Boston

N = 68
N = 40
N = 163
N = 271

- Single Gene Disorders
- Aneuploidy
- Chrom Rearran

Santolaya, Wilkins-Haug et al., ACMG 2008
Patient Declining PGS

- N = 9 (22%)
- N = 23 (34%)
- N = 53 (32%)
- N = 85 (30%)

- Single Gene
- Aneuploidy
- Chrom Rearrange
### ACOG: Recommended Genetic Screening Tests

#### Aneuploidy & Congenital anomalies & Perinatal complications

**Marker Utility Window**

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<tr>
<td>Free Beta</td>
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<td>PAPP-A</td>
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<td>AFP</td>
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#### Ethnic Groups

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Conditions</th>
<th>Risks</th>
</tr>
</thead>
</table>
| **African-American**| Sickled Cell<br>Cystic Fibrosis<br>Beta-Thalassemia | 1 in 10  
|                    |                                                  | 1 in 65  
|                    |                                                  | 1 in 75  |
| **Ashkenazi Jewish**| Gaucher disease<br>Cystic Fibrosis<br>Tay-Sachs disease<br>Dysautonomia<br>Canavan disease | 1 in 15  
|                    |                                                  | 1 in 26 - 1 in 29 |
| **Asian**          | Alpha-Thalassemia<br>Beta-Thalassemia            | 1 in 20  
|                    |                                                  | 1 in 50  |
| **European American**| Cystic Fibrosis                                 | 1 in 25 - 1 in 29 |
| **French Canadian, Cajun**| Tay Sachs disease                              | 1 in 30  |
| **Hispanic**       | Cystic Fibrosis<br>Beta-Thalassemia             | 1 in 46  
|                    |                                                 | 1 in 30 - 1 in 50 |
| **Mediterranean**  | Beta-Thalassemia<br>Cystic Fibrosis<br>Sickle Cell | 1 in 25  
|                    |                                                 | 1 in 29  
|                    |                                                 | 1 in 40  |
### Expanded Screening available to the Ashkenazi Jewish Population

<table>
<thead>
<tr>
<th>Condition</th>
<th>Carrier Frequency</th>
<th>Condition</th>
<th>Cost</th>
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</thead>
<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>1:26</td>
<td>Tay Sachs – enzyme</td>
<td>$140</td>
</tr>
<tr>
<td>Tay-Sachs disease</td>
<td>1:30</td>
<td>Tay Sachs – DNA</td>
<td>$284</td>
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<tr>
<td>Canavan disease</td>
<td>1:57</td>
<td>Canavan</td>
<td>$284</td>
</tr>
<tr>
<td>Familial dysautonomia</td>
<td>1:30</td>
<td>Cystic fibrosis</td>
<td>$430</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>1:100</td>
<td>Familial dysautonomia</td>
<td>$308</td>
</tr>
<tr>
<td>Fanconi anemia (Group C)</td>
<td>1:89</td>
<td>Four conditions recommended by ACOG</td>
<td>$1446</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>1:15</td>
<td>Gaucher</td>
<td>$284</td>
</tr>
<tr>
<td>Glycogen storage disease type 1a</td>
<td>1:71</td>
<td>Niemann-Pick</td>
<td>$207</td>
</tr>
<tr>
<td>Maple syrup urine disease (MSUD)</td>
<td>1:81</td>
<td>Bloom</td>
<td>$155</td>
</tr>
<tr>
<td>Mucolipidosis type IV</td>
<td>1:122</td>
<td>Mucolipidosis IV</td>
<td>$384</td>
</tr>
<tr>
<td>Niemann-Pick Type A</td>
<td>1:90</td>
<td>Glycogen storage disease 1a</td>
<td>$308</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maple syrup urine disease</td>
<td>$252</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fanconi Anemia</td>
<td>$155</td>
</tr>
</tbody>
</table>

- Cost of screening for all 11 conditions: $3191

*Screening should be offered to all couples in which one partner has at least 1 Ashkenazi Jewish grandparent.

*Screening should be offered to the partner with the Ashkenazi Jewish ancestry for most risk reduction benefit.*
### Cystic Fibrosis Carrier Screening

Chance of Dx of mutations depends on Ethnicity and # mutations tested
(Platforms for 23 vs 32 vs 98 mutations; 23 recommended by ACMG)

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Carrier Frequency/ Detection%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>1:25/ 88.2%</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>1:26/ 94%</td>
</tr>
<tr>
<td>African American</td>
<td>1:65/ 64.5%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1:46/ 71.2%</td>
</tr>
<tr>
<td>Asian American</td>
<td>1:90 / 48.9%</td>
</tr>
<tr>
<td>Jewish, non-Ashkenazi</td>
<td>Varies by country of origin</td>
</tr>
<tr>
<td>Other or Mixed ancestry</td>
<td>Varies by country of origin</td>
</tr>
</tbody>
</table>

CF is multisystem genetic disease in which abnormal chloride metabolism across membranes causes dehydrated secretions (thick sticky mucous in lungs/pancreas) and high sweat chloride. Over 1300 mutations in CF transmembrane conductance regulator (CFTR) on chromosome #7.

**Treatment** with pancreatic enzymes, high fat, high carb diet, respiratory therapy with chest percussion/ Inhaled therapy/ DNAse and antibiotics. Median survival: 40 years. Males are infertile with congenital bilateral absence of the vas deferens.
Fragile X screening?

“Genetic counseling and Informed consent for carrier testing can be challenging”

- **Males with a pre-mutation (55-199 repeats)** are at risk for FXTAS a fact he may not want to know

- **Females with a pre-mutation** face decisions concerning:
  1) The risk for full mutation in her progeny
  2) When and how to get pregnant due to increased risk for POF

**Risk for expansion of CGG premutation to full mutation (>200 repeats):**
- 55-59 repeats: 3.7%
- 70-79 repeats: 31.1%
- 80-89 repeats: 57.8%
- 90-99 repeats: 80.1%
- >100 repeats: 100%

Fragile X syndrome (FXS) is the 2nd most common form of inherited mental retardation (Down syndrome is the 1st). Birth rate of 1:4,000 males (1:8,000 females) across all ethnic backgrounds caused by expansion in the **FMR-1** gene.

March of Dimes: www.modimes.org
GeneTests: www.genetests.org
Info gene: www.infogenetics.org
Genetic support groups: www.geneticalliance.com
Metabolic screening: http://genes-rus.uthscsa.edu
Fragile-X Syndrome

“Q. is population screening appropriate”

In a 2009 survey of physician geneticists and genetic counselors, about 60% supported newborn screening for FXS but were less supportive of identifying adult carriers. When asked to choose the best screening model, 29% selected pre-conception, 43% high-risk populations and 28% did not endorse screening at this time.


ACMG and a joint statement by the Child Neurology Society and the American Academy of Neurology recommend test in children with unexplained delays. NSG Counselors and a multicenter working group of genetic counselors test possibly affected individuals followed by cascade testing of extended family members once a target individual has been confirmed. AGOG test any child with developmental delay, autism, or autistic behavior and family; and offer carrier testing to women <40 years old with ovarian failure or an increased follicle-stimulating hormone level. The American Academy of Pediatrics has no formal position on FX testing!
Spinal Muscular Atrophy
Concerns with population screening

- SMN - 1
- SMN - 1
- SMN - 1

Non-Carrier of SMA

- SMN - 1

Carrier of SMA

- SMN - 1

Carrier of SMA

There are 4 (some suggest 5) clinical types of SMA
- Type 1 is lethal in infancy
- Types 2 and 3 are lethal in childhood/young adulthood
- Type 4 has onset in 4th or 5th decade with normal life expectancy

**SMA:** A.R. disease of progressive motor weakness from degeneration and loss of anterior horn cells (lower motor neurons) in the spinal cord and brain stem. SMN1 and SMN2 involved (~95% Hz deletions of exons 7 and 8 in SMN1 and 4-5% have a deletion on one chromosome and a point mutation on the other!
Hemoglobin Stoichiometric assembly

HbA  
\[ \alpha_2 \beta_2 \]  
Hb A  
Adult  
97 %  
Newborn  
20 %

HbF  
\[ \alpha_2 \delta_2 \]  
Hb A2  
2.5  
<0.5

HbA₂  
\[ \alpha_2 \gamma_2 \]  
Hb F  
<1  
80

Fetal cells (RBC, Lymphocytes, Cytotrophoblast)  
in maternal circulation =1/100,000-10,000,000  (Nucleated=Genotyping)

Kleihauer-Betke, 1957  
HbF resists Acid Elution
Mutations in Hemoglobin α and β genes

Inherited mutations leading to:

1) **Quantitative** abnormality of α or β globin synthesis (Thalassemia's)

2) **Qualitative** aminoacid substitution in β globin leading to abnormal function

240,000,000 carriers and 200,000 affected Newborns worldwide each year
# Carrier Rate of Inherited Hemoglobin Disorders

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Sickle Cell Trait</th>
<th>Beta Thal Trait</th>
<th>Alpha Thal Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>West African</td>
<td>1:6</td>
<td>1:50</td>
<td>1:30</td>
</tr>
<tr>
<td>African American</td>
<td>1:12</td>
<td>1:75</td>
<td>1:30</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caribbean, West Indian</td>
<td>1:12</td>
<td>1:50-1:75</td>
<td>1:30</td>
</tr>
<tr>
<td>Southern European</td>
<td>1:30-1:50</td>
<td>1:20-1:30</td>
<td>1:30-1:50</td>
</tr>
<tr>
<td>Northern European</td>
<td>rare</td>
<td>rare</td>
<td>rare</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>rare</td>
<td>rare</td>
<td>rare</td>
</tr>
<tr>
<td>Hispanic Caribbean</td>
<td>1:30</td>
<td>1:75</td>
<td>variable</td>
</tr>
<tr>
<td>Hispanic Mexican, Central American</td>
<td>1:30-1:200</td>
<td>1:30-1:50</td>
<td>variable</td>
</tr>
<tr>
<td>Asian</td>
<td>rare</td>
<td>1:50</td>
<td>1:20</td>
</tr>
<tr>
<td>Southeast Asian</td>
<td>rare</td>
<td>1:30</td>
<td>1:20</td>
</tr>
<tr>
<td>Asian Subcontinent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(India, Pakistan), Middle Eastern</td>
<td>1:50-1:100</td>
<td>1:30-50</td>
<td>variable</td>
</tr>
</tbody>
</table>
# Microcytic Anemia

<table>
<thead>
<tr>
<th>RBC (3.7-5.3 x10E6/uL)</th>
<th>Inherited Hemoglobin Disorder</th>
<th>Iron Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>N or ‡</td>
<td></td>
<td>‡ ‡</td>
</tr>
<tr>
<td>MCV (79-97 fL)</td>
<td>‡ ‡</td>
<td>‡</td>
</tr>
<tr>
<td>MCH (26.6-33 pg)</td>
<td>‡</td>
<td>‡ ‡</td>
</tr>
<tr>
<td>MCHC (31.5-35.7 g/dL)</td>
<td>‡</td>
<td>‡ ‡</td>
</tr>
<tr>
<td>Serum Fe (35-155 ug/dL)</td>
<td>N or ‡</td>
<td>‡</td>
</tr>
<tr>
<td>Ferritin (15-150 ng/mL)</td>
<td>N or ‡</td>
<td>‡</td>
</tr>
<tr>
<td>% Saturation (15-55)</td>
<td>N or ‡</td>
<td>‡</td>
</tr>
<tr>
<td>TIBC (250-450 ug/dL)</td>
<td>N or ‡</td>
<td>‡</td>
</tr>
</tbody>
</table>
### Hb Electrophoresis

<table>
<thead>
<tr>
<th></th>
<th>Alpha Thal Carrier</th>
<th>Beta Thal Carrier</th>
<th>Sickle Cell Carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCV</strong></td>
<td>&lt;80</td>
<td>&lt;80</td>
<td>&lt;80</td>
</tr>
<tr>
<td><strong>Hb Type</strong></td>
<td>AA</td>
<td>AA</td>
<td>AS</td>
</tr>
<tr>
<td><strong>Hb A2</strong></td>
<td>&lt;3%</td>
<td>&gt;4%</td>
<td>&gt;4%</td>
</tr>
<tr>
<td><strong>Hb F</strong></td>
<td>Normal</td>
<td>Elevated</td>
<td>Elevated</td>
</tr>
</tbody>
</table>
WWW Sites of Interest

- Joint Center for Sickle Cell and Thalassemic Disorders: http://www-rics.bwh.harvard.edu/sickle/ (Overview of sickle cell disease, thalassemia and iron kinetics)

- The Sickle Cell Information Center, Emory University: http://www.emory.edu:80/PEDS/SICKLE/ (Includes PowerPoint presentations on sickle cell disease)
CURRENT MANAGEMENT OF BLOOD DISORDERS

a) Normal gene cloned into a plasmid/virus, the gene must be associated with regulatory elements and inserted into the affected person’s cells

b) Compatible cell based therapy

In-utero THERAPY?
Case Study

Documentation of Family History:
Genetic Consultation & Reproductive Options

Aunt 59 heart attack

47,XY,+21 by Karyotype report

Brother 10
Breast Cancer

Mom 52

Sister 26

Dad 59

Healthy

Husband 28

34 healthy

Adopted
Consultand

Sister 34

8

The patient's brother in law: Intelligence quotient test (IQ~50) with cognitive impairment, Repaired ventricular septum defect, and recurrent ENT infections

The patient has genetic consultation at 13+w gestation and opts for 1st Trimester Screening Test NT-US measurement + NIPS
Prenatal Diagnosis of Genetic Disorders

- Amniocentesis
- CVS
- PUBS

US-guided risks

- Amniocentesis: ~2%
- CVS: ~0.3%
- PUBS: ~1%

Weeks: 11, 16, 20, 40 (Week)
The reported Risk for miscarriage after CVS in twins ranges between 2 and 11%.

Clinical case:
maternal age (34yo) NIPS + for T21 reported at 15w. The patient opts for a Diagnostic amniocentesis.

FISH: a quick targeted approach at 2MB DNA loci

No need for Microarray in this case

AFP for NTD/Abdominal wall defects

Prenatal Diagnosis:
US-guided CVS and Amniocentesis

Procedure related risk for CVS and Amniocentesis depends on Gestational age, Chorionicity and Experience of the operator!
In summary

Information is not knowledge
Opinion is not scientific evidence
Personal Genome Project (PGP)

2008: Announcement of the $5,000 Genome

*Genomes for ALL*

Next-generation technologies that make reading DNA fast, cheap and widely accessible are coming in less than a decade.

Their potential to revolutionize research and bring about the era of truly personalized medicine means the time to start preparing is now.

By George M. Church

Scientific American, 2006

<table>
<thead>
<tr>
<th>Launch</th>
<th>Platform</th>
<th>List Cost</th>
<th>Counselor</th>
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</thead>
<tbody>
<tr>
<td>deCODEme</td>
<td>Nov-07</td>
<td>Illumina</td>
<td>$985</td>
</tr>
<tr>
<td>23andMe</td>
<td>Nov-07</td>
<td>Illumina</td>
<td>$399</td>
</tr>
<tr>
<td>Navigenics</td>
<td>Apr-08</td>
<td>Affymetrix</td>
<td>$2500+$250 annual sub</td>
</tr>
<tr>
<td>SeqWright</td>
<td>Jan-08</td>
<td>Affymetrix</td>
<td>$998</td>
</tr>
</tbody>
</table>

Bio-IT World November, 2008
Physician’s have Scientific, Social and Economic responsibilities to Patient’s and to the Health Care system

- They provide counseling, order tests, interpret tests, offer management plans and treat patients

- Considerations for an Ethic-based practice in the “Omics” age
  - Testing children for adult-onset disorders?
  - How do we respond to unanticipated information?
  - Premarital Testing? Successful use in communities that adhere to strict cultural rules rather than as part of a modern USA culture.
OMICS = Overall application of biotechnologies & informatics to enhance screening, diagnosis and to define the cellular metabolic pathways and networks governing biological processes and diseases.
“Patients can differentiate their feelings about the diagnosis from how the diagnosis was given”

Thank You For your Attention!