Prenatal Diagnosis

• Using a wide variety of screening and diagnostic tests to assess health of a fetus to:
  – Manage the pregnancy
  – Determine potential outcomes
  – Plan for complications at birth
  – Decide whether to continue the pregnancy
  – Discover conditions that may impact future pregnancies
The goal of prenatal diagnosis is not to generate perfect babies.

“The are no perfect human specimens - we are all genetically flawed in some way.”

- F.Collins
The goal of prenatal diagnosis is to help parents learn what *they* need to know about the health of their unborn child to help them make informed decisions for themselves and their family within the context of their own value system.
General Caveats about Prenatal Diagnosis

- All couples have ~3% risk of having a child with congenital problems requiring intervention
- No 100% guarantees - even if prenatal tests are ‘normal’
- All couples bring unique ethnocultural, moral, and/or religious perspectives to the process
- Use of non-judgmental, non-directive genetic counseling is important in helping families make the best choice for them
- The decision to terminate or continue a pregnancy based on prenatal diagnostic findings is never an easy decision
Goals of Prenatal Diagnosis and Counseling

• Assess pregnancy
• Determine specific risks to fetus
• Evaluate prenatal diagnostic options
• Educate family about diagnosis, likely outcomes, potential and management options
• Discuss risks, benefits, and uncertainties
• Explore family concerns
• Provide risk assessment for other family members
• Provide psychosocial support and follow-up
Who benefits from prenatal diagnosis?

- Older women (≥ 35) at increased risk of chromosome disorders
- Individuals in populations at increased risk of a genetic disease:
  - Tay-Sachs: Ashkenazi Jews, French Canadians
  - Sickle cell anemia: Africans, Mediterraneans, Arabs, Turks, Indo-Pakistanis
  - Thalassemias: Mediterraneans, Arabs, Turks, Indo-Pakistanis, Southern and Southeast Asians
  - Cystic Fibrosis: Caucasians
  - Fragile X syndrome: All women (?)
- Family history of a genetic disease/chromosome disorder
- Maternal disease associated with increased risk of birth defects (diabetes, phenylketonuria)
- Known teratogen exposure during pregnancy
- Abnormal screening tests or ultrasounds
- Women who are concerned/worried
Preconception/Carrier Testing

- Couples/individuals in “high risk” populations considering pregnancy should be offered voluntary, informed testing prior to pregnancy.
- Appropriate education and counseling about risks and benefits of tests and various reproductive options should be available prior to and after testing.
Prenatal Diagnosis Techniques

• Maternal Serum Screening Tests
  – Triple screen (alpha-fetoprotein, beta-HCG, and estriol) for neural tube defects and chromosome trisomies

• Visualization of the fetus
  – Ultrasound - 2D and 3D
  – Other (very special circumstances - X-ray, fetoscopy)

• Genetic and biochemical studies of fetal cells
  – Amniocentesis
  – Chorionic villus sampling
  – Fetal blood sample (percutaneous umbilical sample)
  – Circulating fetal cells in maternal blood
Maternal serum alpha-fetoprotein (MSAFP)

• Levels increase with gestational age in amniotic fluid and cross placenta into maternal bloodstream
• With neural tube (anencephaly, spina bifida) and body wall defects (gastroschisis, omphalocele) AFP is HIGH
• Using MSAFP along with detailed ultrasound study is sensitive to detect open body wall and neural tube defects
• MSAFP is LOWER in trisomies but using MSAFP alone to pick up trisomies is not sensitive or specific
• MSAFP most sensitive between 16-18 weeks
• To interpret must know gestational age, twin status, maternal health status(diabetes), and race - falsely high and falsely low values are often due to poor gestational dating
NIPT Non Invasive Prenatal Test
ccffDNA circulating cell-free fetal DNA

Cell-free Fetal DNA

Blood sample from pregnant mother

Cell-free DNA fragments from the mother and fetus (placenta)

Plasma

White Blood Cells

Red Blood Cells

NIPD = Non Invasive Prenatal Diagnosis
Figure 2. Noninvasive Prenatal Diagnosis with the Use of Plasma Cell-free Fetal RNA or DNA in Maternal Blood Derived from Dying Trophoblast Cells of the Placenta.

For cell-free fetal RNA, target RNA molecules containing SNPs are quantified with the use of a PCR assay. The allelic ratio, which is determined on quantitative PCR, is used to determine the chromosome copy number when the fetus is heterozygous for the SNP. A 1:1 ratio of amplified allelic variants is expected in the euploid state, whereas a ratio of 2:1 indicates trisomy. Cell-free fetal DNA in plasma can be sequenced directly because it is smaller than maternal cell-free DNA; it can also be enriched by means of size fractionation before DNA sequence analysis. The abundance of specific chromosome sequences can be compared with normal reference samples or with another chromosome as a specified denominator in the sample to determine variation in chromosome copy number or structural changes in chromosomes. Additional methods of analysis of cell-free fetal DNA to determine chromosome copy number have been described, including methods to quantify differentially methylated regions of specific fetal chromosomes with the use of PCR. These methods await validation.
Sequencing of short fragments for NIPT

- **Fetal cell-free DNA**
- **Maternal cell-free DNA**

**Non-pregnant women**

- chr17
- chr18
- chr19
- chr20
- chr21
- chr22

**Pregnant women No trisomy 21**

- chr17
- chr18
- chr19
- chr20
- chr21
- chr22

**Pregnant women Trisomy 21**

- chr17
- chr18
- chr19
- chr20
- chr21
- chr22
Diana W. Bianchi, MD

Title(s)
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Department + Services
Pediatrics, Genetics and Metabolism

My Expertise
Prenatal diagnosis, fetal malformations, fetal treatment, reproductive genetics

Meet Diana W. Bianchi, MD

Boston
Ultrasonography

- Non-invasive - no known risks to mother or fetus
- 2-D, 3-D high resolution and fetal echocardiograms
- Assess fetal proportions, sex, position, growth; placenta, amniotic fluid
- Accurately estimate fetal age
- At 6 weeks can see developing embryo
- Between 16-20 weeks gestation is optimal time to screen for congenital anomalies for prenatal diagnosis
- False positive and false negative findings - conditions with subtle findings may be missed, (eg. trisomy 21)
Gastroschisis
Some conditions detected by ultrasound

- Neural tube defects
- Body wall defects
- Major organ abnormalities
- Oligo- or polyhydramnios
- Major limb abnormalities
- Growth disturbances
Amniocentesis & Chorionic Villus Sampling

(a) Amniocentesis

(b) Chorionic villus sampling
Chorionic Villus Sampling

- Invasive technique to obtain fetal cells
- Study chromosomes, DNA, or biochemical profile of fetus
- Most often performed between 10-13 weeks gestation
- More genetic material from cells to study right away
- Risks:
  - Fetal loss rate slightly higher than amnio - about 1%
  - Very slight risk of increased limb abnormalities if done < 10 weeks
  - Risk of infection
Amniocentesis

• Invasive technique to obtain fetal cells
• Study chromosomes, DNA, or biochemical profile of fetus
• Approach via mother’s abdomen under ultrasound guidance
• Enough fluid after 14 weeks of gestation to perform safely
• Most often preformed between 15 and 20 weeks gestation
• Risks:
  – fetal loss - < 0.5% higher than normally expected
  – trauma and infection,
  – risk of club foot reported when done < 13 weeks
• Later in pregnancy (eg. third trimester), amniotic fluid can be taken to assess fetal lung maturity prior to a premature delivery
Percutaneous Umbilical Blood sampling

• Invasive procedure to obtain fetal blood cells
• Study chromosomes, DNA, blood chemistries, or biochemical
• Needle under ultrasound guidance to obtain blood from umbilical vein
• Risks:
  – Fetal loss rate higher than amnio or CVS (at least 2% mid-2nd trimester)
• Rarely needed except in special circumstances where results cannot be obtained by amniocentesis or CVS techniques
Indications for Offering Amniocentesis or Chorionic Villus Sampling

- Advanced maternal age
- Abnormal maternal serum marker test
- Family history of chromosome abnormality
- Genetic disease detectable by biochemical or DNA analysis
- Concerns of patient
Prenatal genetic testing is a process, not just a laboratory procedure

- Pre-testing evaluation, education, genetic counseling, and informed consent
- Laboratory analysis
- Accurate interpretation of results
- Follow-up must include support, education, and management
Every pregnancy should be assessed for risk of birth defects

- Obtain family history of birth defects or genetic disorders
- Determine if there recurrent pregnancy losses?
- Look for signs of fetal abnormalities - IUGR, poly- or oligohydramnios?
- Offer screening for NTDs, aneuploidy
- Offer screening for age and ethnicity based increased risks
- Minimize risk with optimal preconception care, prenatal care and avoidance of teratogenic agents
- Check for maternal illnesses or exposures
High Fetal Risk
Pregnancy Management

• Conduct appropriate diagnostic studies and genetics evaluation as needed
  – Chromosome, biochemical, molecular studies...
  – Consults

• Look for associated malformations
  – Ultrasounds, echocardiograms...

• Carefully discuss diagnostic, prognostic, and therapeutic issues and options with parents as non-directively as appropriate
Management After Loss of a Fetus due to Miscarriage and Termination

- Conduct clinical evaluation/autopsy to confirm diagnosis
- Offer parents an opportunity to see fetus if miscarriage, still birth or late termination due to genetic problems
  - Name, photograph, obtain hair, memorialize, bury...
- Provide referrals to social work/psychological services and support groups as appropriate
- Arrange follow-up genetic counseling
- Most importantly be aware, available, and sensitive to needs - all people will deal loss in different ways
Primum non nocere

“I will apply treatment for the benefit of the sick according to my ability and judgment; I will keep them from harm and injustice”

3rd paragraph
Physician’s Hippocratic Oath
HUMAN EMBRYOLOGY

- **PRE-EMBRYO** (wks 0-2)

- **EMBRYO** (wks 2-8)

- **FETUS** (wks 8-38)

- **EDD (Expected Date of Delivery)**
  
  38 wks from conception
  
  (40 wks [280 days] from LMP)
  
  (Final dating by US)
Pre-natal vs Pre-implantation diagnosis
Ovulation induction
Fertilization

- Conventional Insemination
- Intracytoplasmic Sperm Injection (ICSI)
PGD Process

- Ovulation Induction
- Retrieval
- Fertilization
- Embryo Bx on Day-3
- Genetic Analysis
- Embryo Transfer
Preimplantation genetic diagnosis (PGD) is the process of performing genetic testing before embryos created by in vitro fertilization (IVF) are transferred. A biopsy is performed to remove one cell (or possible more) from each developing embryo. Polar body biopsy can be used to test for aneuploidy or maternally-inherited conditions. Blastomere or trophoblast biopsy can be used for aneuploidy, recessive, or paternally-inherited conditions. Genetic testing, such as fluorescence in situ hybridization (FISH), microarray, sequencing, or linkage analysis, can then be performed while the embryos are stored. PGD is most often used to test for familial translocations, single-gene disorders, and aneuploidy. The results are used to select the apparently unaffected embryos for transfer. PGD has less than a 100% accuracy rate and prenatal diagnosis is recommended to confirm that a pregnancy is unaffected. PGD can also be used to select for a certain gender, HLA type, or other genetic trait that is desired.
DEFT TOUCH: Technicians manipulate egg cells
Figure 1: **Cleavage-stage biopsy**
PGD. Trasferimento dei pre-embrioni non affetti
FISH – Based Single-Cell Preimplantation Sexing (Severe X-linked Conditions)
What’s in the Future?

With the advent of the microarray techniques for the analysis of the genome, transcripts of thousands of genes can be tested at one time, and the combination of both might dramatically change our future.