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OB-GYN BOARD REVIEW
2018

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HERE, THERE, AND EVERYWHERE

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GENETICS
THE BASICS

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BASELINE GENETIC RISK

• 3-5% of live births will have a major birth defect and 10% will have a minor congenital abnormality
• Down Syndrome (Trisomy 21)
  • Most common trisomy of live births
    • 1/800 live births
DEFINITIONS

• Aneuploidy
  • Haploid gamete or diploid cell lacks expected number of chromosomes - n or 2n
• Trisomy
  • An additional chromosome is present
    • Meiotic non-disjunction in Meiosis I
• Polysomy
  • Additional chromosome is a sex chromosome

• Monosomy
  • 2n - 1
• Polyploidy
  • 3N = 69
  • Usually caused by dispermy
• Mosaicism
  • 1 individual has 2 or more genotypes from the same zygote
  • Different cells have a different chromosomal makeup
  • True effect depends not only on how many cells affected but which cells are affected
• Chimerism
  • 1 individual has 2 or more genotypes from different zygotes
  • Two non-identical twin embryos merge together
  • May have patches of non-matching DNA

• Deletions
  • The chromosome breaks and a piece is lost
  • Involves loss of genetic information
  • Can be considered a “partial monosomy”
• Inversions
  • Two breaks in one chromosome - fragment inverted and rejoined within the chromosome
  • Rearrangements usually do not involve loss of genetic material
  • Individuals usually have a normal phenotype
  • Paracentric – Inverted area includes centromere
  • Pericentric – Inverted area does not include centromere
EXAMPLES

- Deletion
  - 46,XX,del(1)(q24q31)
    - Female with a deletion of chromosome 1 on the long arm (q) between bands q24 to q31

INVERSIONS

- Paracentric Inversion
  - 46,XY,inv(3)(p12p33)

- Pericentric Inversion
  - 46,XY,inv(3)(p23q27)

TRANSLOCATIONS

- Robertsonian translocations
  - Exchange of short arms of the acrocentric chromosomes
    - 13, 14, 15, 21, and 22
  - Considered balanced
- Reciprocal translocations
  - Two non-homologous chromosomes break and exchange fragments
MEIOSIS FACTS

- Difference in meiosis I is the most unique
- Difference in female and male gamete formation
  - Female meiosis I arrested in Prophase I (dictyotene) in oocyte development at 5 months embryonic age
  - Meiosis I completed at ovulation
  - Meiosis II completed at fertilization

MEIOSIS FACTS

- Meiosis I
  - Pairing and crossing over of two chromatids of homologous chromosomes
  - Crossing over is necessary for subsequent separation of homologues
  - Nondisjunction
    - Decreased numbers of crossovers
    - Aging affects function of the spindle apparatus of the oocyte

MULTIFACTORIAL DISORDERS

- 1/1000 risk
- 2-3% if one affected sibling
- 4-6% if 2 affected sibs
- Most often anatomic abnormalities
- Traits/disorders affected by multiple factors
  - Genetic and environmental
  - NTD's
  - Hydrocephaly
  - Cleft lip
  - Cardiac Disorders
  - Diaphragmatic hernia
  - Posterior urethral valves
  - Renal agenesis
  - Pyloric stenosis
  - Omphalocele
PREGNANCY LOSS

- First Trimester
  - Aneuploidy - 40-60%
  - Trisomy - 50%
  - 16 most common
  - 45, X - 25%
  - Triploid - 20%
  - Unbalanced translocation (3%)

- Second Trimester
  - 10-30% aneuploid
  - Trisomy 13
  - Trisomy 18
  - Trisomy 21
  - 45, X
  - Stillbirths
  - Trisomy 18 (10%)

MODES OF INHERITANCE

PEDIGREE SYMBOLS
AUTOSOMAL DOMINANT INHERITANCE

- Manifests in the heterozygote
- Equal in males and females
- Vertical inheritance through generations
- Affected parents have 50% risk that offspring will be affected

Penetrance

- Some individuals with disease-associated genotype do not develop the disease
- An all-or-none phenomenon

Variable expression

- The variable severity of a genetic trait
- Different individuals show different degrees of disorder
- All develop the disease in some manner but to varying degrees

AUTOSOMAL DOMINANT DISORDERS

- Achondroplasia
- Huntington Chorea
- Neurofibromatosis
- Marfans
- Familial Polyposis
  - Normal Parents
  - New mutation
  - Form Fruste - subclinical in parent

EXAMPLES
AUTOSOMAL RECESSIVE INHERITANCE

- Manifests only in homozygotes
- Parents of affected individual both heterozygous (carriers) are phenotypically normal
- Often present among sibs but not usually transmitted through generations
- Offspring of heterozygous parents
  - 1 in 4 chance of being affected
- Ratio of affected to normal offspring is 1:3
- Pedigree typically shows two or more affected sibs in one family but other relatives normal
  - “Horizontal” inheritance

EXAMPLES

- Sickle cell disease
- PKU
- Tay Sachs
- Cystic Fibrosis
X – LINKED DOMINANT INHERITANCE

- Trait never passed from father to son
- All daughters of affected male and a normal female are affected
- All sons of an affected male and a normal female are normal
- Matings of affected females and normal males
  - 1/2 sons affected
  - 1/2 daughters affected
- Males usually more severely affected than females
  - May be lethal in males
- Females more likely to be affected than males even if disease not lethal in males
25. **X-LINKED DOMINANT INHERITANCE**

- Vitamin D-resistant rickets

26. **X-LINKED DOMINANT INHERITANCE**

27. **X-LINKED RECESSIVE INHERITANCE**

- Occurs only in males (with few exceptions)
  - Transmitted from carrier females to affected males
  - All affected males in a family related through their mothers
  - Male to male transmission excludes X-linked inheritance
  - Male offspring of affected male all normal and female offspring all carriers
  - Affected female extremely rare
    - Daughter of an affected male and a carrier female
  - Criss-cross pattern of inheritance
    - Disease appears in uncles and nephews
  - Not transmitted in direct lineage - appears to “skip a generation”
    - Typically affected grandfather through carrier daughter to half of his grandsons
X -LINKED RECESSIVE INHERITANCE

- Hemophilia
  - Inability to form blood clots due to deficiency of factor VIII
  - About 1 in 10,000 males
- Christmas disease
  - Due to deficiency of factor IX
  - X-linked but at a different locus from that of factor VIII
- Duchenne muscular Dystrophy
  - Affects males
  - Muscular degeneration/weakness begins age of 3 to 5 years, wheelchair bound by teen years, and can die in early 20s from respiratory failure
  - Deficiency of the protein dystrophin in skeletal muscle, caused by a mutation of the dystrophin gene
- Becker muscular dystrophy
  - Affects males
  - Weakness begins in adolescence, progresses slowly and is not lethal

X -LINKED RECESSIVE INHERITANCE

- Red-green color blindness
  - Inability to distinguish red from green
  - Mutation affecting one or the other gene causes partial color blindness - mutations affecting both genes cause total color blindness
- Glucose-6-phosphate dehydrogenase (G-6PD) deficiency
  - Affects primarily Africans, Asian, Middle Eastern and Mediterranean descent
  - Enzyme helps to produce glutathione
  - Protects cells from oxidative stress
  - RBC's exposed to oxidative stress undergo hemolysis due to reduction in glutathione
  - Oxidative stressors
    - Foods
    - Infections
    - Drugs

EXAMPLES
Mitochondrial inheritance:

- Mitochondrial DNA contains ~ ten genes involved in oxidative phosphorylation
  - Capable of mutation
  - Leber optic atrophy - classic example of a disease
- Ovum has ~ 100,000 copies of mitochondrial DNA
- Sperm has fewer than 100 copies – likely lost at fertilization
- Essentially all of our mitochondria come mother
- Affected fathers produce no affected offspring
- Offspring of affected mothers are all affected
MITOCHONDRIAL INHERITANCE

Affected males do not transmit the trait to any of their children.

Affected females transmit the trait to all of their children.

EXAMPLES

- Leber's hereditary optic neuropathy (LHON)
- Mitochondrial encephalomyopathy
- Myoclonic epilepsy and ragged-red fibers (MERRF)
- Leigh syndrome, subacute sclerosing encephalopathy
- Kearns-Sayre syndrome (KSS)
- Myoneurogenic gastrointestinal encephalopathy (MNGIE)
- Luft Disease

IMPRINTING

- For some genes, the origin of the gene is important.
- Gene inherited from father acts differently from that inherited from mother.
- Uniparental Disomy:
  - Rare chromosomal event in which both chromosomes come from a single parent.
- Example:
  - Small deletion of long arm of chromosome 15 while the homologous chromosome remains intact (15q11-13).
  - Deletion of paternal origin results in Prader-Willi Syndrome.
  - Deletion of maternal origin results in Angelman Syndrome.
- Two genetic syndromes have very different clinical findings.
37 **IMPRINTING - EXAMPLES**

**PRADER-WILLI**
- Neonatal hypotonia
- Developmental deficiency
- Severe obesity
- Short stature
- Hypogonadism
- Mild to moderate mental retardation
- Small hands and feet

**ANGELMAN**
- Ataxia, seizures
- Hyperactivity
- Severe mental retardation
- Absence of speech
- Inappropriate laughter
- "Happy puppet" syndrome

38 **MULTIFACTORIAL INHERITANCE**

- Most affected children have normal parents
- Recurrence risk increases with number of affected children
- Recurrence risk increases with severity of the defect
  - More severely affected parent more likely to produce an affected child
- Consanguinity slightly increases risk for affected child
- Risk of affected relatives falls with degree of relationship
- If two sexes have different probability of being affected, least likely sex - if affected - most likely sex to produce an affected offspring

39 **TRANSLOCATIONS**

- Robertsonian translocations
  - Exchange of short arms of the acrocentric chromosomes
    - 13, 14, 15, 21, and 22
    - Considered balanced
- Reciprocal translocations
  - Two non-homologous chromosomes break and exchange fragments
Individual Aneuploidies and Syndromes

A Review

Down Syndrome

- Mental retardation
- Nuchal skin fold thickening
- Cardiac defects – Atrioventricular septal defect
- Hypotonia
- Flat facies
- Slanted palpebral fissures
- Small ears
- Hyoplasia of midphalanx of fifth finger
- Wide iliac wings
Trisomy 21 Risks

- Higher risk in twin pregnancy
  - Two fetuses = double risk
- Patient of age 33 years with twin gestation equal risk of patient with a singleton 35 years old
  - Down syndrome risk: 1/445
  - Any chromosomal anomaly: 1/176

Trisomy 18 (Edward’s Syndrome)

- 1/8000 live births
- Clenched fists
- Rocker bottom feet
- Micrognathia
- Cardiac abnormalities
- Short sternum
- Severe mental retardation
- Single umbilical artery
- 30% die 1st month
- 50% die 2nd month
- < 10% survive 1yr
- Female 3:1
- Recurrence < 1%
- Primary nondisjunction (47, XY +18)

Trisomy 13 (Patau’s Syndrome)

- 1/20,000 live births
- Cleft lip and/or palate
- Omphalocele
- IUGR
- Holoprosencephaly
- Single umbilical artery
- Primary nondisjunction
  - Recurrence almost 0 if neither parent has (47,+ 13)
- 50% die 1st month
- < 3% live to age 1
Cri du chat (5p-)

- Deletion in short arm chromosome 5
- 1/20,000 live births
- High pitched monotonous cry
- Round face
- Marked epicanthal folds
- Mental retardation

Turner’s Syndrome (45, X)

- 1/2500 live births
- Sporadic
- Most frequent chromosomal abnormality
- 7% of spontaneous abortions
- Cystic hygromas
- Anasarca
- Renal anomalies
- Coarctation of aorta
- Primary amenorrhea
- Short stature
- Webbed neck
- No hair, breasts
- Gonadal dysgenesis
- E2 & Test Low
- FSH, LH high

Turner Syndrome

- 2% of recognized 45,X embryos survive to term
  - 98% are lost
- Result of non-disjunction
  - Can occur at either meiotic division in either spermatogenesis or oogenesis
  - 80% result of paternal nondisjunction
  - Can also result from early mitotic nondisjunction
    - Mosaic 46,XX/45,X
Klinefelter's Syndrome (47,XXY)

- 1 in 850 to 1 in 500 live births
- Pubic and axillary hair
- Scant facial hair
- Tall body habitus
- Female fat distribution
- 20 fold increase in risk of breast cancer

Klinefelter’s Syndrome

- Child must get the Y chromosome from his father
- Nondisjunction in either meiotic division in mother
- Only occur in the first meiotic division in father
- If nondisjunction occurred in father, zygote would have to get both X and Y chromosome in same sperm
  - In spermatogenesis this could only result from a mistake in first division
  - Second meiotic division of spermatogenesis separates either the two X or two Y chromatids into different gametes

Gender Chromosome Abnormalities

- XYY syndrome
  - 1/1000 male births
  - Nondisjunction in second meiotic division of spermatogenesis
  - Found in almost 1/50 males in prison populations
  - Aggressive behavior and less intelligence than siblings
- XXX syndrome (47,XXX)
  - Very normal phenotype
  - As number of X chromosomes increases mental deficiencies increase
**Cystic Fibrosis**
- 1/2500 live births
- Carrier 1 in 22 to 1 in 30 Caucasians
- Autosomal recessive
- Delta F 508 on Chromosome 7 most common mutation
  - Many other mutations
  - Not all mutations identified
- Non-affected sibling of patient with CF has a 2/3 chance of being a carrier
- ACOG recommends offering screening to everyone – not just Caucasian individuals

**McCune Albright Syndrome**
- Polyostotic Fibrous Dysplasia
- Café au le spots
- Autonomous endocrine hyperfunction
  - Precocious puberty
  - Gonadotropin independent
- Skeletal dysplasia
  - Fibrous dysplasia
  - Hypophosphatemia

**Neural Tube Defects**
- Polygenic Inheritance
- 1 in 2000 risk
  - Decreased from 1 to 2 per 1000 following the supplementation of foods with folic acid
- Recurrence 2-3%
  - Multifactorial
Sickle Cell Disease

- Autosomal Recessive
- 8 percent (1 in 12) of African Americans are carriers
- 0.15% of African Americans have SS
- 2 parents with trait
  - 25% risk of an affected child
  - Risk of SS for African American
    - $1/12 \times 1/12 \times 1/4 = 1/576$

Rokitansky-Kuster-Hauser

- Mullerian Agenesis
- 1/4000 births
- 46, XX
- Vaginal agenesis
- Normal ovarian function
- Normal growth & development
- 1/3 have urinary tract abnormalities
- Need karyotype
  - Distinguish between testicular feminization
- Primary amenorrhea

Testicular Feminization (Androgen Insensitivity)

- Male pseudohermaphrodite
- 46XY
  - X-linked recessive
  - 25% risk of affected child
  - 25% risk of carrier
  - End-organ insensitivity to androgens
  - Blind vagina - no uterus
  - Absent to sparse hair
- Body responds normally to antimullerian hormone
- Testosterone & LH increased
- 5% risk of malignant tumors
- Remove gonads after puberty
Kallman Syndrome

- Gonadotropin Deficiency
- Hypogonadotropic hypogonadism
- Anosmia
- Color blindness
- Cleft lip & palate

Omphalocele

- 1/5800 live births
- Associated with trisomy 13 and trisomy 18
  - 35-58%
- 30% association with other fetal anomalies
- Umbilical cord inserts into center of sac
- 30-40% mortality
- Recurrence
  - Less than 1%

Gastroschisis

- 1/10,000 live births
- Vascular compromise umbilical vein, omphalomesenteric artery
- No significant increase in other fetal anomalies
  - 10 percent
- Cord is connected to abdominal wall
Ethnic Genetic Disorders

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<td>African Americans</td>
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<td>Breast Cancer</td>
<td>African Americans</td>
<td>1 in 8</td>
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<td>Colon Cancer</td>
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Definitions

**Screening Test**
- Offered to a large, low-risk group
- Identifies smaller group who may benefit from further testing
- Inexpensive
- Low risk to patient
- Easy

**Diagnostic Test**
- Offered to high-risk group
- Provides a definitive result
- Expensive
- Higher risk to the patient
- More difficult to perform
Elements of Genetic Counseling

- Time
- Sometimes requires a known diagnosis
- Communication
  - Information gathering
  - Patient/family education
- Non-directive counseling
  - An objective discussion
  - Probably unrealistic
- Important to know limitations of knowledge and refer appropriately

General Introduction

- Importance of prenatal genetic screening
  - Reassurance
    - Majority of patients undergo screening or diagnostic testing for this reason
  - Prepare for a child with special needs
  - Therapeutic alteration of outcome (rare)
  - Terminate pregnancy

Increased Genetic Risk

- Pregnant women
- Advanced maternal age
- Certain ethnic groups
  - Ashkenazi Jews
  - French Canadians
  - Cajun
- Previous affected fetus
- Fetus with a major sonographic anomaly
- Men/women carrying chromosomal abnormalities
  - Translocation
  - Inversion
  - Aneuploidy
Factors Affecting Genetic Risk

- Environment
- Genetics
- Socioeconomic status
- Maternal diabetes
- Maternal weight
- Maternal alcohol use
- Family History of NTD
- Medication exposures

Common Cytogenetic Abnormalities

- Trisomy 21
  - 1 in 800 live births
- Trisomy 18
  - 1 in 8000 live births
- Trisomy 13
  - 1 in 20000 live births

- 45,X
  - 1 in 10000 live births
- 47,XXX; 47,XXY; 47,XYY
  - 1 in 900 live births
- Del(5p)
  - 1 in 20000 live births

Methods of Genetic Screening

- Maternal serum screening
- Ultrasound
- Combinations of the above
- Circulating Cell Free DNA analysis
- Microarray
Methods of Genetic Screening

- Microarray
  - Identifies chromosomal abnormalities including submicroscopic abnormalities too small to be detected by conventional karyotyping
  - Fetal DNA obtained from chorionic villus sampling or amniocentesis
  - Potential for complex results and clinically uncertain findings
    - Can result in substantial patient anxiety

Methods of Genetic Screening

- Fetus with major structural abnormalities
  - Chromosomal microarray analysis – replaces fetal karyotype
- Structurally normal fetus undergoing invasive prenatal testing
  - Either fetal karyotype or chromosomal microarray
- Should not be restricted to women aged 35 years and older
- Intrauterine fetal demise when cytogenetic analysis desired
  - Chromosomal microarray analysis on fetal tissue
- Not recommended for evaluation of 1st or 2nd losses
- Comprehensive patient pretest and posttest genetic counseling from a genetic counselor or geneticist essential
- Should not be ordered without informed consent

Effectiveness of a Screening Test

- Detection rate
  - How many affected pregnancies will be identified by the test
- False positive rate
  - Number of unaffected pregnancies that screen positive by the test
- Odds of being affected given a positive result (OAPR)
  - Combines detection rate, false positive rate and disease prevalence into one ratio
  - Allows comparison of the number of diagnostic procedures required to identify one affected pregnancy using each screening test
ACOG Committee Opinion

October 2015 Number 643

• Identification and referral of maternal genetic conditions in pregnancy
  • Management of genetic conditions is complex and may require a multidisciplinary approach
  • Patients with genetic conditions or at risk of a genetic condition, should have a preconception evaluation with their obstetrician–gynecologists, genetics specialists, maternal–fetal medicine specialists, or other appropriate subspecialists
  • Patients with established causative mutations for a genetic condition, and who desire prenatal genetic testing, should be offered preimplantation genetic testing with in vitro fertilization
  • Once pregnant, patient with a genetic condition should have her initial prenatal examination early in 1st trimester for coordination of prenatal screening/testing and evaluation of pregnancy risks.

Ethics of Prenatal Screening

ACOG Committees on Ethics and Genetics

• Genetic testing of the fetus offers opportunities and ethical challenges
  • Preconception and prenatal genetic screening and testing recommended for a limited number of severe child-onset diseases
    • May pursue assisted reproductive technology to avoid conception of an affected child
    • To detect and treat a fetus with a condition in utero
    • To consider termination of a pregnancy
    • To prepare for the birth of a child with special needs
  • With advancing genetic technology requests for testing of fetuses for less severe child-onset conditions, adult-onset conditions, or genetically linked traits will increase
    • Testing fetuses for adult-onset disorders with no known therapeutic or preventative treatment (save prevention by pregnancy termination) should raise caution
    • Consideration should be given to interest individuals may have in terminating a pregnancy that may result in a life that they feel morally obliged or prefer not to bring into the world
    • Referral to parent support networks, counselors, social workers, or clergy may provide additional information and support

Ethics of Prenatal Screening

ACOG Committees on Ethics and Genetics Recommendations

• Identify patients who are candidates for genetic testing and maintain competence in the face of increasing genetic knowledge
• Recognize geneticists and genetic counselors are important part of health care team and should consult and refer as needed
• Discuss with patients importance of genetic information for their kindred
  • Recommend information be shared with potentially affected family members as appropriate
• Be aware that genetic information has potential to lead to discrimination in workplace and affect individual’s insurability
  • Physicians have an obligation that includes a mandate to prevent discrimination
    • Advocacy for legislation to ban genetic discrimination
“Ideally, all women should be offered aneuploidy screening before 20 weeks of gestation, regardless of maternal age.”

“A strategy that incorporates both first- and second-trimester screening should be offered to women who seek prenatal care in the first trimester.”
CATEGORIES OF STUDY EVIDENCE

• I - Evidence obtained from at least one properly designed randomized controlled trial.
• II-1 Evidence obtained from well-designed controlled trials without randomization.
• II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.
• II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments also could be regarded as this type of evidence.
• III Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

GRADING OF RECOMMENDATIONS

• Based on the highest level of evidence found in the data
  • Level A—Recommendations are based on good and consistent scientific evidence.
  • Level B—Recommendations are based on limited or inconsistent scientific evidence.
  • Level C—Recommendations are based primarily on consensus and expert opinion.

ACOG PRACTICE BULLETIN
MAY 2016 NUMBER 163

• Prenatal genetic screening
  • Assessment of risk of patient having a fetus affected by a genetic disorder
• Prenatal genetic diagnostic testing
  • Determines with as much certainty as possible whether a specific genetic disorder or condition is present in the fetus
  • Fetal chromosomes evaluated for presence/absence of abnormalities in chromosome number, deletions, and duplications, or fetal DNA evaluated for specific genetic disorders
  • No one screening test is superior to another in all test characteristics
  • Screening should be an informed patient choice that fits the clinical circumstances, values, interests, and goals
Chromosomal abnormalities occur in 1 in 150 live births.

- Prevalence greater earlier in gestation as aneuploidy accounts large number of early pregnancy loss.
- Factors which increase risk of fetal aneuploidy:
  - Increasing maternal age
  - History of a prior aneuploid fetus
  - Presence of fetal anomalies

Autosomal trisomies almost common aneuploidies

- Down syndrome (trisomy 21) most common
  - 1 in 800 live births
- Most common form of inherited intellectual disability
- 95% of cases of Down syndrome result from nondisjunction involving chromosome 21

Most common sex chromosome aneuploidy

- Klinefelter syndrome (47,XXY)
  - 1 in 500 males
- Only viable monosomy is Turner syndrome (45,X)

Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy.

- Choice to perform screening or diagnostic testing depends on the woman’s goals, values, and desire for informational accuracy.

Screening identifies two groups

- Positive screen result with an increased risk of fetus with an aneuploidy
  - Offered counseling and diagnostic testing
- Negative screen result with lower posttest probability of evaluated aneuploidies
  - Counseled regarding an adjusted lower risk
  - Should not be offered additional screening tests - increased risk of false positives
  - May be offered diagnostic test if additional findings occur
• Counseling
  • Patient should be counseled regarding the adjusted likelihood of carrying a fetus with the evaluated aneuploidies.
  • Potential for fetus to be affected by genetic disorders not evaluated by the screening or diagnostic test
  • Prenatal diagnosis of fetal aneuploidy
    • Expectant management
      • Education for preparedness and expectations
      • Delivery at a tertiary care center
      • Potential need for hospice
      • Termination of pregnancy
    • Expectant management with special needs adoption

• Minor markers
  • Nonspecific physical characteristics more common in fetuses with Down syndrome than those without
    • Common in unaffected fetuses as well
    • Increased nuchal skinfold
    • Highest risk of aneuploidy
    • Isolated echogenic intracardiac focus
    • Lowest risks of fetal aneuploidy
    • Isolated presence of minor marker
    • Analyte screening or cell free DNA should be offered

• Major limitations of second-trimester ultrasonographic markers
  • Lack of standardization in measurements and characteristics that define a

• Level A Recommendations
  • Negative screening test result should not be offered additional screening tests for aneuploidy
  • Increase their potential for a false-positive test result
  • Enlarged nuchal translucency an obvious anomaly, or a cystic hygroma identified on ultrasonography
  • Genetic counseling and diagnostic testing for aneuploidy
  • Follow-up ultrasonography for fetal structural abnormalities
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• Level A Recommendations
  • Enlarged nuchal translucency or cystic hygroma and normal fetal karyotype
  • 2nd trimester anatomic evaluation, fetal cardiac ultrasonography and counseling regarding possible genetic syndromes not detected by aneuploidy screening
  • Following a 1st trimester screen:
    • 2nd trimester assessment for open neural tube defects and other structural defects
    • Serum MSAFP and/or fetal sonogram
  • Cell-free DNA is a screening test and should not be used as a substitute for diagnostic testing
  • Positive cell-free DNA test result should be confirmed by a diagnostic test prior to any irreverable action
  • Non-reportable or indeterminate (no call result) cell free DNA screen should undergo counseling due to an increased risk of aneuploidy

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• Level B Recommendations
  • Cell-free DNA screening for microdeletions has not been validated and is not recommended
  • Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during their pregnancy
  • No method of aneuploidy screening is as accurate in twin gestations as in singleton pregnancies
    • Analysis screening for fetal aneuploidy should be limited to singleton and twin pregnancies

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• Level C Recommendations
  • Screening for aneuploidy should be an informed patient choice
  • Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit
  • All women should be offered the option of aneuploidy screening or diagnostic testing regardless of maternal age
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• Level C Recommendations
  • Isolated minor marker for aneuploidy
  • Offer aneuploidy screening if not yet offered
  • Positive result from traditional screening
    • Cell-free DNA versus definitive testing
      • May delay definitive diagnosis to identify aneuploidy fetus
    • Parallel/simultaneous testing with multiple screening options
      • Not cost-effective and should not be performed.
  • Multifetal gestations, fetal demise, or in one fetus
    • Serum-based aneuploidy screening should be discouraged
    • Increased risk for inaccurate test result in these circumstances

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• Cell-free DNA is a screening test
  • Should not be used as a substitute for diagnostic test
  • Positive predictive value
    • 95% for Down syndrome,
    • 64% for trisomy 18
    • 44% for trisomy 13
    • 39% for sex chromosome aneuploidy
    • Positive screen could represent confined placental mosaicism, a resorbing twin or a maternal malignancy or maternal aneuploidy

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• Cell-free DNA is a screening test
  • More effective with higher fetal fractions
    • Sampling before 10 weeks’ gestation
    • High maternal body mass index.
    • Fetal aneuploidy
      • 8% failed to obtain a result, and 32% of those were aneuploid
Cell free DNA
- Amount increases throughout gestation
- Cleared from maternal circulation within hours after childbirth
- Several molecular methods developed to analyze cell-free DNA
  - All appear to have similar detection and false-positive rates
- Determine fetal sex, identify presence of Rh-positive fetus, and detect some paternally derived autosomal dominant genetic abnormalities
- Screening from 10 weeks’ gestation until term
- Highest reported detection rate for Down syndrome
  - 96% detection with positive screening rates of less than 0.5% among women with a reportable result
  - Managing women with no reportable result as screen positive will decrease specificity and increase positive screening rate for this testing.

Biochemical Markers and Genetic Screening

Prenatal Genetic Screening
- Maternal serum screening
  - 1st trimester
  - 2nd trimester
  - Combinations
  - Circulating cell free fetal DNA
- Prenatal sonography
  - 1st trimester
    - Nuchal translucency
    - Nasal bone
  - 2nd trimester
    - Nuchal fold
    - Minor markers
    - Cardiac defects
Quad Screen

- Geared to the detection of Down syndrome
- Most common chromosomal abnormality in live births
  - 1 in 700 to 1 in 800 live births
- 95% trisomy 21
- 3% translocations
- 2% mosaic
- Phenotype variable
- Trisomy 18
- Open neural tube defects
- Smith-Lemli-Opitz

Factors Taken into Account

- Gestational age
- Maternal weight
- Maternal race
- Maternal insulin-dependent diabetes
- Multiple fetal pregnancy
- Family history of Down syndrome

Trisomy 21
**Maternal Serum Screening**

**Quad Screen**

- Trisomy 18
  - Low AFP, Estriol, and HCG

- Smith-Lemli-Opitz
  - Abnormality of cholesterol synthesis resulting in increased levels of 7-dehydrocholesterol
  - Due to absence of 7-dehydrocholesterol reductase

- Majority of babies with trisomy 21 born to women under the age of 35 years

- Maternal serum screening most accurate between 16-18 weeks' gestation
  - Can be drawn from 15 – 22 6/7 weeks’ gestation

**Alpha-Fetoprotein (AFP)**

- Produced in fetal liver and GI tract
- Excreted in fetal urine
- Rises throughout pregnancy
- First protein routinely measured in the maternal blood
- High levels originally used to identify fetuses at risk for neural tube defects
- 1984 correlation of low AFP levels with fetuses with Down syndrome
Serum Alpha-Fetoprotein (MSAFP)

- **High**
  - Normal variant
  - Open neural tube defects
  - Abdominal wall defects
  - Bleeding
  - Fetal demise
  - Kidney disorders
  - Oligohydramnios
  - Multifetal gestation
  - Under estimated fetal age
  - Increase in 3rd trimester complications

- **Low**
  - Normal variant
  - Chromosomal trisomies
  - Gestational trophoblastic disease
  - Fetal death
  - Over estimated fetal age

Serum Proteins

- **Human Chorionic Gonadotropin (HCG)**
  - Placental glycoprotein
  - Peaks at 10 weeks' and then gradually declined
  - High levels associated with increased risk for Down syndrome
  - HCG and free beta HCG
    - Able to be utilized in both second and first trimesters

Serum Proteins

- **Estriol**
  - Produced from interaction of the placenta, fetus, and mother
  - Increases throughout pregnancy
  - Low levels associated with increased risk for Down syndrome
  - Addition to AFP and HCG resulted in the “triple test”
    - Detection rate of about 65% for 5% false positive rate
  - Addition showed only a small incremental
Serum Proteins

• Inhibin A
  • Produced by the placenta
  • Addition to the “triple screen” resulted in the “quad screen”
    • Can detect 70-75% of cases of fetal Down syndrome
    • High levels associated with Trisomy 21

Quad Screen - Summary

• Alpha-fetoprotein (AFP)
  • Protein produced by the fetus’ liver
    • Decreased in trisomy 21

• Unconjugated Estriol (UE)
  • Produced in the placenta, fetus liver, and input from maternal liver
    • Decreased in trisomy 21

Quad Screen - Summary

• Human Chorionic Gonadotrophin (hCG)
  • Produced by the placenta
    • Elevated in trisomy 21

• Inhibin-A
  • Hormone produced by the placenta
    • Elevated in trisomy 21
Maternal Serum Screening

- Limitations to 2nd trimester screening
  - Earliest can be performed ~15 weeks
  - 25% of Down syndrome cases not identified
  - 60 amnios need to be done for single case of Down syndrome
  - 1 normal fetus lost for every three DS fetuses identified
  - Interest in earlier screening methods

Maternal Serum Screening

- Penta Screen
  - Adds hyperglycosylated hCG (invasive trophoblast antigen) to the Quad Screen
  - Detection rate not formally studied
  - Limited data to assess accuracy when compared to other maternal screens
  - Not commonly used

Methods of Screening

- First Trimester Serum Screening
  - Combination of free βhCG, PAPP-A, and maternal age
    - PAPP-A: Pregnancy Associated Plasma Protein-A
      - Produced by the placental trophoblast
      - Detection rate ~ 60% with 5% false positive rate
    - Independent of other markers
      - Allows addition of other markers to alter the calculated risk
    - Down syndrome detection rates ~ 73% to 84% (FPR of 5%)
Potential Etiologies
• Aortic isthmic narrowing
• Other fetal cardiovascular defects
• Abnormalities in the extracellular matrix
• Abnormal or delayed development of the lymphatic system
• Specific etiology may vary with the underlying condition

10 – 13 6/7 weeks’ gestation
• Crown-rump length 45 (38) and 84 mm
• Sagittal view of the fetus in a horizontal position with profile visible
• Neck in a neutral position
• Image fills 75% of the screen
• Widest part of translucency is measured
• Measurements taken with calipers placed inner border to inner border of the nuchal translucency at its widest point
The thicker the nuchal translucency, the greater the risk.
Nuchal Translucency


• Nuchal translucency greater than or equal to 3.0 mm considered screen positive
  • NT measurement of 3.0 mm - minimal benefit in waiting for serum screening results
  • No benefit for NT of 4.0 mm or greater
  • Serum screening is not necessary
  • Genetic consultation should be offered
  • Fetal echocardiogram should be considered

Nuchal Translucency and Congenital Heart Abnormalities

• The thicker the nuchal translucency, the greater the risk
  • Additionally, the greater the nuchal translucency – the greater the risk of adverse pregnancy outcome in general

Types of Screening

• Integrated screening
  • First- and second-trimester combined test
  • Patient receiving a single risk assessment after second test
  • Can be performed with or without nuchal translucency
  • High rates of non-adherence (25% without reminder)
  • Highest detection rate

• Independent sequential screening
  • Obtain 2 individual results from 1st trimester then 2nd trimester testing without incorporating information from 1st trimester test
  • Has unacceptably high false-positive rate
  • NOT RECOMMENDED
Types of Screening

- Step-wise sequential screening
  - First-trimester screen and prenatal diagnosis if calculated risk above a specific cutoff
  - If patient screens below risk cutoff, she is offered second-trimester screening and receives a combined result

- Contingent screening
  - Groups women into a high, low, or intermediate risk categories after first-trimester screen with management dependent on first-trimester risk assessment
  - Most cost-effective

Screening for open Neural Tube Defects

- Following 1st trimester screening
  - MSAFP in second trimester
    - Drawn as a single order
    - Not part of a Quad screen
  - Ultrasound
  - Both

Current Analyte Screens

- Performance of current screening tools for T21 detection
- All sensitivity data taken in a fixed 5% positive screen rate
Prenatal Genetic Screening

<table>
<thead>
<tr>
<th>1st TRIMESTER SCREEN</th>
<th>2ND TRIMESTER SCREEN</th>
<th>3RD TRIMESTER SCREEN</th>
<th>FULL INTEGRATED SCREEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP less than 5th percentile</td>
<td>Blood sample</td>
<td>Blood sample</td>
<td>Blood sample</td>
</tr>
<tr>
<td>Elevated hCG, AFP, dimeric inhibin A</td>
<td>Blood sample</td>
<td>Blood sample</td>
<td>Blood sample</td>
</tr>
<tr>
<td>Fetal death</td>
<td>Blood sample</td>
<td>Blood sample</td>
<td>Blood sample</td>
</tr>
</tbody>
</table>

- First trimester: PAPP A less than 0.4 MOM (5th percentile) and/or low HCG (less than 0.5 MOM) in first trimester
  - Spontaneous fetal and neonatal loss
  - Fetal growth restriction
  - Preeclampsia
  - Placental abruption
  - Preterm delivery

- Other Clinical Implications
  - Elevated hCG, AFP, dimeric inhibin A
  - Fetal death
  - Fetal growth restriction
  - Preeclampsia
  - Likelihood of adverse pregnancy outcome increases with the increasing number of minor markers
Other Clinical Implications

- Second trimester
  - Elevated hCG or AFP, or dimeric inhibin A
    - Fetal death
    - Fetal growth restriction
    - Preeclampsia
  - Decreased MSAFP (less than 0.25 MOM) or estriol (less than 0.5 MOM)
    - Fetal death
    - Fetal growth restriction
    - Preeclampsia
  - Estriol less than 0.3 MOM
    - Genetic consultation for Smith-Lemli-Opitz

Other Clinical Implications

- Combination of a placenta previa and elevated MSAFP in the second or third trimester has been associated with placenta accreta

  - Likelihood of adverse pregnancy outcome increases with the increasing number of minor markers

Noninvasive Prenatal Screening

- Noninvasive prenatal testing uses cell-free short fragments of DNA isolated from plasma of pregnant women
  - Testing should be an informed patient choice after pretest counseling - should not be part of routine prenatal laboratory assessment
  - Should not be offered to low-risk women or women with multiple gestations because not been sufficiently evaluated
  - Negative test result does not ensure an unaffected pregnancy
  - Patient with a positive test result should be referred for genetic counseling and offered invasive prenatal diagnosis
  - Does not replace accuracy and diagnostic precision of CVS or amniocentesis
Noninvasive Prenatal Screening

• Indications (OLDER OPINION)
  • Maternal age 35 years or older at delivery
  • Fetal ultrasonographic findings indicating an increased risk of aneuploidy
  • History of a prior pregnancy with a trisomy
  • Positive screening result for aneuploidy - first trimester, sequential, or integrated screen, or quad screen.
  • Parental balanced robertsonian translocation with increased risk

Circulating Cell Free DNA (cfDNA)

• Short fragments of DNA found in maternal blood
  • Cell-free DNA fragments from both mother and fetus
  • Maternal blood obtained after 10 weeks’ gestation
  • Relative amount of free DNA assessed in relation to maternal controls
    • Determines chance fetus has trisomy 21, 18 or 13 based on relative amount of DNA from chromosomes 21, 18 and 13
    • Can also assess abnormalities of the X and Y chromosomes
  • Results usually available within 10 to 14 days
  • Accuracy of cell-free DNA testing not validated in low-risk women

Circulating Cell Free DNA (cfDNA)

• cfDNA originates from placental cells undergoing apoptosis releasing fetal cells in circulation
  • Circulating fetal DNA in maternal plasma greater than 10%
  • Positive predictive value lower in the general population versus high risk patients
    • Lower prevalence of aneuploidy in a low risk population
  • Cell free DNA assessment for microdeletions not validated and not recommended
Circulating Cell Free Fetal DNA Isolation

Methods of Analysis

<table>
<thead>
<tr>
<th>Directed Analysis</th>
<th>Massively Parallel Shotgun Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directed analysis of cfDNA fragments ensuring reproducible results</td>
<td>Random analysis of cfDNA fragments</td>
</tr>
<tr>
<td>Low cost due to analysis of one-tenth as many cfDNA fragments as shotgun sequencing</td>
<td>High cost due to analysis requiring tens of millions of cfDNA fragments</td>
</tr>
<tr>
<td>Detailed test result that provides individualized trisomy risk assessment for each patient sample</td>
<td>Binary test result with no specific individual risk score or quantifiable information</td>
</tr>
</tbody>
</table>
Current Offerings

- MaterniT21 PLUS
- Verify
- Progenity
- Nifty
- Harmony
- Panorama
- VisibiliTi

Summary of Detection Rates Trisomy 21

Screening in Multifetal Gestations

- Affected by number of fetuses and zygosity
- No method of aneuploidy screening is as accurate as in singleton gestation
- Nuchal translucency measurements allow each fetus to be screened independently
- Single enlarged nuchal translucency in monozygotic twins may be early sign of twin to twin transfusion syndrome
- Cell free DNA not recommended in twin gestation
- Serum based screening not recommended if there is a fetal demise or fetal anomaly in one twin due to risk of inaccurate results
Comparison of the Screening Modalities

- Society for Maternal-Fetal Medicine Consult Series recommendations (March 2017)
  - Sonogram for nuchal translucency in a patient with negative cfDNA screen is not recommended
  - Diagnostic testing should not be recommended in patients with only an isolated soft marker and a negative cfDNA screen
    - Finding should be described as not clinically significant or as a normal variant
  - Isolated soft marker and negative 1st or 2nd trimester screen should be described as not clinically significant or a variation of normal
  - Patients with a fetus with a structural abnormality identified by ultrasound should be offered diagnostic testing with chromosomal microarray
  - Routine screening for microdeletions with cfDNA is not recommended

Cell free DNA (cfDNA) Screening and Role of Ultrasound

- Society for Maternal-Fetal Medicine Consult Series recommendations (March 2017)
  - Sonogram for nuchal translucency in a patient with negative cfDNA screen is not recommended
  - Diagnostic testing should not be recommended in patients with only an isolated soft marker and a negative cfDNA screen
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  - Patients with a fetus with a structural abnormality identified by ultrasound should be offered diagnostic testing with chromosomal microarray
  - Routine screening for microdeletions with cfDNA is not recommended
The Role of Ultrasound
Introduction

- Least effective primary screening for trisomy 21
  - Detects 50 to 60 percent of affected fetuses
  - Should not be used in isolation to confirm or exclude trisomy 21

- Rate of aneuploidy directly related to number of anomalies identified
- Specific anomaly may provide clues to the underlying aneuploidy
- Major structural anomalies vs minor markers
  - Minor marker
    - Structural change that may be transient and when in isolation is thought to have little pathologic significance
    - May be seen more frequently in chromosomally abnormal fetuses
- Examples

Characteristics of Down Syndrome

- "Mongoloid" features
- Learning difficulties
- Delayed development
- Reduced muscle tone (hypotonia)
- Broad hands with short fingers
- Clinodactyly
  - Little finger curves inwards
- Deep cleft between 1st & 2nd toe
- Long crease on side of foot
- Single palmer crease
  - Eyes slant upwards & outwards
  - Extra epicanthic fold
  - Back of neck flatter with loose skin
  - Flat nasal bridge
  - Smaller oral cavity with protruding tongue
  - Below average length at birth
Characteristics of Down Syndrome

- Congenital cardiac defects
- Chest & sinus problems
- Feeding difficulties
- Gastrointestinal problems
- Thermoregulation
- Tongue control
- Vision & hearing problems

- Alzheimer's disease
- Leukemia
- Epilepsy
- Thyroid disorders
- Mean life expectancy 50-55 years

Major Ultrasound Findings and Associated Risks

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Aneuploidy Risk</th>
<th>Associated Aneuploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic Hygroma</td>
<td>60 to 75%</td>
<td>45X, 21, 18, XXY</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>3-8%</td>
<td>13, 21, Triploidy</td>
</tr>
<tr>
<td>Cardiac Defects</td>
<td>5-30%</td>
<td>21,18,13,22,8,9,</td>
</tr>
<tr>
<td>Complete AVSD</td>
<td>40-70%</td>
<td>21</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>30-40%</td>
<td>13,18</td>
</tr>
<tr>
<td>Hydrops</td>
<td>30-80%</td>
<td>13,21,18,45X</td>
</tr>
</tbody>
</table>

Cystic Hygroma
Cystic Hygroma

Hydrocephalus

The Normal Heart
### Specific Minor Markers

**Thick Nuchal Fold**
- First sonographic marker associated with DS
- Measured 15 - 20 wks
- ≥ 6 mm
- Identifies 40% of fetuses with DS
- Single most specific and sensitive marker for DS
- Confers highest risk of aneuploidy

**Echogenic Bowel**
- 0.2 to 1.4% scans
- Normal variant
- Fetal aneuploidy
- IUGR
- Cystic fibrosis
- Bleeding
- Congenital infection

**Renal Pyelectasis**
- 0.3 to 4.5%
- > 4 mm AP diameter
- PPV 1 in 340
- AP diameter varies over time
- Association greatest when other anomalies seen
- Clinical relevance may lie in post-delivery concerns
**Specific Minor Markers**

- **Echogenic Intracardiac Focus**
  - 4% of scans
  - Highest in Asians; lowest in Blacks
  - Calcifications of papillary muscle of unknown etiology
  - Can be single or multiple
  - Most are in the left ventricle
  - Original studies in high risk populations
  - In low risk population does not increase risk significantly
  - Carries lowest risk for aneuploidy

- **Choroid Plexus Cyst**
  - 1% incidence
  - Can be single or multiple
  - Associated with trisomy 18 (Not trisomy 21)
  - High risk group – 1 in 128
  - Low risk group – 1 in 189
  - Recent studies show risk low when heart and hands appear normal >/= to 18 weeks

- **Nasal Bone**
  - Most recent addition
  - 1st trimester
    - 73% of fetuses with trisomy 21 vs 0.5% of normal fetuses
    - Unable to obtain in 6% of cases
  - 2nd trimester controversies
    - Absence vs Presence vs Hypoplastic
    - Nasal bone length ratios
    - Interpretation varies by ethnic group
Specific Minor Markers

- Other markers
  - Clubfoot
  - Single umbilical artery
  - Dangling of the choroid plexus with normal atrial dimension
  - Iliac angle widening
  - Hypoplasia middle phalanx 5th digit
  - Clinodactyly
  - Delayed fusion of chorion/amnion
  - Sandal-gap toe

Statistics Review

- Positive predictive value
  - Chance that a positive test will end up in an affected fetus

- Negative predictive value
  - Chance that a fetus will be normal and is not affected

- Likelihood ratio (positive)
  - How much more likely is a fetus with a specific finding to have a chromosomal abnormality than one without the finding

- Likelihood ratio (negative)
  - How much more likely is absence of a finding in a normal fetus vs an affected fetus
Statistics and Prenatal Diagnosis

- **Sensitivity** - true positives
  - How well the test identifies fetuses with aneuploidy
- **Specificity** - true negatives
  - How well the test identifies fetuses with normal chromosomes
- **Positive Predictive Value**
  - Chance that a positive test will result in an affected fetus
- **Negative Predictive Value**
  - Chance that a negative test will result in a normal fetus
- **Likelihood Ratio (positive)**
  - How much more likely a fetus with a specific finding will have a chromosomal abnormality than one without the finding
- **Likelihood Ratio (negative)**
  - How much more likely is absence of a finding in a normal fetus vs an affected fetus

<table>
<thead>
<tr>
<th>Finding</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural Defect</td>
<td>25</td>
</tr>
<tr>
<td>Nuchal Thickening</td>
<td>18.6</td>
</tr>
<tr>
<td>Echogenic Bowel</td>
<td>5.5</td>
</tr>
<tr>
<td>Short Humerus</td>
<td>2.5</td>
</tr>
<tr>
<td>Short Femur</td>
<td>2.2</td>
</tr>
<tr>
<td>Echogenic Intracardiac Focus</td>
<td>2</td>
</tr>
<tr>
<td>Renal Pyelectasis</td>
<td>1.6</td>
</tr>
<tr>
<td>Normal Ultrasound</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Risk Modification (Example)

- Maternal age 35 years = 1/270 risk for trisomy 21
- 1st trimester screen places risk at 1 in 450
- Ultrasound shows bilateral pyelectasis
  - Likelihood ratio of 1.6
- $\frac{1}{450} \times 1.6 = 1$ in 281
- Still a low risk result
Minor Marker Follow-up

- Third trimester follow-up
  - Renal pyelectasis
  - Echogenic bowel
  - Short femur and/or humerus
- No follow-up necessary
  - Choroid plexus cyst
  - Echogenic bowel
- Detailed counseling
  - Hypoplastic or absent nasal bone
  - Echogenic bowel
  - Thickened nuchal skinfold

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Minor Marker for Aneuploidy

Summary

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Table 1: Management of Fetal Anomalies Related to Prenatal

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal pyelectasis</td>
<td>Consult urology and consider</td>
</tr>
<tr>
<td></td>
<td>referral to pediatric urologist</td>
</tr>
<tr>
<td>Echogenic bowel</td>
<td>Obtain neonatal urology referral</td>
</tr>
<tr>
<td>Short femur and/or humerus</td>
<td>Evaluate growth and development</td>
</tr>
<tr>
<td>Choroid plexus cyst</td>
<td>Obtain ophthalmology referral</td>
</tr>
<tr>
<td>Echogenic bowel</td>
<td>( N/A ) for echogenic bowel</td>
</tr>
<tr>
<td>Thckened nuchal skinfold</td>
<td>( N/A ) for thckened nuchal</td>
</tr>
</tbody>
</table>

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Definitions

A Screening Test
- Offered to a large, low-risk group
- Identifies smaller group who may benefit from further testing
- Inexpensive
- Low risk to the patient
- Easy

A Diagnostic Test
- Offered to a high-risk group
- Provides a definitive result
- Expensive
- Higher risk to the patient
- More difficult to perform

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Prenatal Diagnostic Testing for Genetic Disorders
- Intended to determine whether a specific genetic disorder or condition is present in the fetus
  - Amniocentesis
  - Chorionic villus sampling (CVS)
  - Fetal imaging with ultrasonography, echocardiography, or magnetic resonance imaging may be diagnostic of a particular structural fetal abnormality that is suggestive of an underlying genetic condition.

Objective
- Detect health problems that could affect the woman, fetus, or newborn
- Provide patient and her obstetric care provider enough information to allow fully informed decision about pregnancy management
- Important that patients understand the benefits and limitations of all prenatal screening and diagnostic testing

Recommendations and conclusions based on good and consistent scientific evidence (Level A)
- Chromosomal microarray analysis
  - Detects pathogenic copy number variant in ~1.7% of patients with normal ultrasound and normal karyotype
  - Made available to anyone choosing to undergo invasive testing
  - Primary test (replacing conventional karyotype) for patients undergoing prenatal diagnosis for fetal structural abnormality detected by ultrasound
  - Identifies 6% of fetuses with normal karyotype
  - If structural abnormality consistent with a particular fetal aneuploidy - FISH may be offered before microarray analysis
  - Early amniocentesis (before 14 weeks' gestation) not recommended
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Recommendations and conclusions based on good and consistent scientific evidence (Level A)
- Abnormal FISH not be considered diagnostic – additional confirmatory finding necessary
- Rate of procedure-related pregnancy loss attributable to a prenatal diagnostic procedure
  - Approximately 0.1–0.3% in procedures performed by experienced health care providers
- Transmission of HIV with amniocentesis not increased in women treated with CART and viral load undetectable

Recommendations and conclusions based primarily on consensus and expert opinion (Level C)
- All pregnant women should be offered prenatal assessment for aneuploidy (screening or diagnostic) regardless of maternal age or other risk factors
- Prenatal genetic testing cannot identify all abnormalities or problems in a fetus
- Genetic testing should be discussed as early as possible in pregnancy, ideally at the first obstetric visit, so that first-trimester options are available

Candidates for Prenatal Diagnostic Testing
- Advanced maternal age
- Microdeletions or duplications do not increase
- Advanced paternal age (40 to 50 years or greater)
- Increased risk for single gene disorders
  - Achondroplasia
  - Apert Syndrome
  - Crouzon Syndrome
- Mutations occur during spermatogenesis
- No recommended screening panels
- Parental carrier of chromosomal rearrangements
  - Risk of unbalanced traslocation
    - 5 to 30 percent if an affected child already born
    - 0.5 percent if identified for other reasons (infertility evaluation)
Candidates for Prenatal Diagnostic Testing

- Parental aneuploidy or mosaicism
  - Increased risk of trisomy in women with trisomy 21
- Prior child with structural birth defects
  - Recurrence risk 2 to 3 percent
  - Most are multifactorial
- Parental carrier of a genetic disorder
  - Sickle cell disease
  - Tay-Sachs
  - Cystic fibrosis
  - Autosomal dominant disorders
  - Diagnosis must be established by molecular testing of an affected child
- Previous fetus or child with autosomal trisomy or sex chromosome aneuploidy
  - Recurrence risk
    - 1.6 to 8.2 x the maternal age related risk of trisomy
    - Unclear level of increase with 47, XXX; 47 XXY
    - Not increased with 45,X
- Structural anomalies identified by ultrasound
  - Increased risk for aneuploidy, copy number variants (deletions, duplications), and genetic syndromes

Diagnostic Prenatal Tests

- Amniocentesis
- Chorionic villus sampling (CVS)
- Percutaneous umbilical blood sampling
- Preimplantation genetics
- Fetal skin sampling
- Fetal tissue biopsy
- Fetoscopy
**Diagnostic Methods**

- **Traditional Amniocentesis**
  - Introduced in the 1950’s
  - 15-20 weeks’ gestation
  - 0.1 to 0.3% fetal loss rate
  - 1% leakage of fluid
  - 0.1% intra-amniotic infection
  - Unclear risk – vertical transmission of maternal HIV, Hepatitis B and C
  - 99% accurate
  - Results 10 to 14 days

- **Early Amniocentesis**
  - 10 to 14 weeks’ gestation
  - 2 to 5% fetal loss rate
  - 3.5% leakage of fluid
  - 1.3% risk of clubfeet
  - Should not be done prior to 13 weeks’ gestation

- **Chorionic villus sampling**
  - Sampling of chorion frondosum
  - Introduced 1968
  - 10-12 weeks’ gestation
  - 1.1 to 1.3% fetal loss rate
  - Limb reduction defects if < 10 weeks’ gestation
  - Transcervical contraindicated with a vaginal infection and maternal blood group sensitization
  - Risk – 0.5%
Diagnostic Methods

- **Percutaneous umbilical cord sampling (Cordocentesis)**
  - Introduced 1973
  - Varying gestational ages
  - 1.4 to 2% fetal loss rate
  - 6.6% risk of bradycardia
  - Cord hematomas
  - Bleeding from puncture site
  - Infection
  - Maternal - fetal bleed

Diagnostic Methods

- **Preimplantation genetics (PID)**
  - Implies IVF
  - 8 cell stage
    - Blastomere day 3
  - Set apart 1-2 cells (embryobiopsy)
  - Tested by FISH
  - Implant embryos with favorable test results
Genetic diagnosis for fetal demise

- Conventional karyotype
  - Can only be obtained with living tissue
  - Higher culture failure rate

- Microarray
  - Does not require viable cells
  - Preferred test
  - Cannot be contaminated with maternal tissue or blood

Mosaicism

- Can occur with chorionic villus sampling
  - Amniocentesis normal 90 percent of time
  - Confined placental mosaicism carries an increased risk for 3rd trimester fetal growth restriction
  - Uniparental disomy (both chromosomes from one parent) testing indicated as follow-up to confined placental mosaicism
    - Trisomy rescue
Obstetric Sonogram and Doppler

Benefits
- Accurate pregnancy dating
- Detection of fetal anomalies
  - 35 to 50 percent of major fetal malformations
    - Varies in different clinical settings and according to the skill of the sonographer

Ultrasound Safety
- Should be performed only with a valid medical indication
- Use lowest possible exposure setting to gain necessary images
  - ALARA principle
    - “As low as reasonably achievable”
- No confirmed damaging biological
  - No fetal harm has been demonstrated in more than 30 years of use
The First Trimester

Advantages
- Diagnosis of abnormal pregnancy
  - Anembryonic gestation
  - Embryonic demise
    - Transvaginal sonogram should identify cardiac motion in an embryo of 5 mm in length
- Multifetal gestation
  - Optimal time to determine chorionicity
  - Best time to evaluate the uterus, adnexal structures, and cul-de-sac

New terminology
- Pregnancy of unknown location
  - Neither an intra or extrauterine pregnancy is identified in the context of a positive beta HCG
    - Very early pregnancy
    - Complete Ab
    - Ectopic pregnancy

Early pregnancy failure
- Absence of a visible yolk sac with mean sac diameter of 13 mm
- Absence of a visible embryo with mean sac diameter of 20 mm
- Absence of cardiac motion with an embryo measuring 5 mm or more in maximal length
- Presence of an empty amnion
- Presence of an adnexal mass in absence of an intrauterine gestational sac may indicate an ectopic pregnancy
The Second Trimester

- Standard Exam
- Specialized Exam
- Limited Exam

The Normal Anatomy Survey

- Fetal Head Measurements (BPD, HC)
Thick Nuchal Fold

- Transaxial plane at level of cavum septi pellucidi and cerebellar hemisphere
- Measured from outer edge of occipital bone to skin surface
- Increases with advancing gestational age
- Measured between 15 and 19 6/7 weeks' gestation
- Thickness ≥ 6 mm considered abnormal

Cystic Hygroma

- Imaging feature of fluid-filled cysts
- Often associated with congenital anomalies
- Requires serial monitoring and intervention if symptomatic
Turner Syndrome

- Cardiac defects
- Cystic hygroma
- Hydrops
- Renal anomalies
- Shortened femur

Trisomy 21

Major Anomalies
- Cardiac defects
- Cystic hygroma
- Duodenal atresia
- Hydrops

Minor Anomalies
- Clinodactyly
- Echogenic intracardiac focus
- Hyperechoic bowel
- Mild ventriculomegaly
- Nasal bone abnormality
- Nuchal thickening >/= 6mm
- Pelvic angle widening
- Renal pyelectasis >/= 4mm
- Sandal gap toe

Trisomy 18

- Agenesis of the corpus
- Arthrogrypotic hands/wrists
- Cardiac defects
- Cerebellar dysgenesis
- Clubbed feet
- Cleft lip and palate
- Cystic hygroma
- Diaphragmatic hernia
- Intrauterine growth restriction
- Microcephaly
- Micrognathia
- Neural tube defects

Minor Anomalies
- Ocular abnormalities
- Omphalocele
- Polyhydramnios
- Radial ray abnormalities
- rocker bottom feet
- Minor markers
  - Brachycephaly/strawberry skull
  - Choroid plexus cysts
  - Limb shortening
  - Single uterine artery
Trisomy 13
- Cardiac defects
- Central nervous system abnormalities
- Cystic hygroma
- Facial abnormalities - cleft lip and palate
- Echogenic kidneys (poly cystic)
- Intrauterine growth restriction
- Holoprosencephaly
- Microcephaly
- Neural tube defects
- Ocular abnormalities
- Omphalocele
- Polydactyly
- Rocker bottom feet
- Minor markers
  - Echogenic intracardiac focus
  - Mild ventriculomegaly
  - Pyelectasis
  - Single umbilical artery
The Lip - Clefting

- Orofacial clefting
  - 60-75% involve cleft lip and palate
  - 25-40% involve isolated cleft lip
- Cleft lip
  - 80% unilateral
  - 2:1 left vs right sided
  - 2:1 male vs females

- Genetics
  - Polygenic, multifactorial inheritance
  - Single gene abnormality – 3%
  - Specific gene candidates being investigated

- Recurrence risk - examples
  - Normal parents and one affected child
    - 4% (cleft lip/palate)
  - One affected parent and one affected child
    - 12% (cleft lip/palate)
  - Both parents affected and one affected child
    - 45% (cleft lip/palate)

The Normal Anatomy Survey
The Ventral Wall

Ascites
- Hydrops
  - Fetal anemia
    - Isoimmunization
    - Parvovirus
    - TORCH infections
  - Isolated
  - Fetal urine
  - Check MCA Doppler

Hydrops
Ventral Wall Defects

Gastroschisis

Omphalocele
**Ventral Wall Defects**

- **Gastroschisis**
  - 1 in 5000 live births
  - Paraumbilical defect involving all layers of abdominal wall
  - Associated GI tract abnormalities
  - 10% other fetal abnormalities

- **Omphalocele**
  - Extrusion of abdominal contents into base of umbilical cord
  - Associated anomalies - 50 to 80 percent
  - Associated chromosomal abnormalities - 40 - 60%
  - Beckwith-Wiedeman syndrome

**Duodenal Atresia**

Most often associated with trisomy 21 but can be seen in chromosomally normal fetuses.

**Sonographic Components**

- Fetal Bony Structure
  - Skull
  - Long bones/extremities
  - Ribs
  - Spine
The Lemon and Banana Signs

- Neural tube defect
- Skull defect
- Normal variant
- Rarely

Lemon Sign

- Skeletal Dysplasia
- Thanatophoric dwarfism
- Craniostenosis
The Spine

Neural Tube Defect

- Myelomeningocele
- Can be open or closed defects
- Open defects will have elevated MSAFP

Sonographic Findings in NTDs

Open neural tube defect
Neural Tube Defect

- Anencephaly
  - Absence major portion of brain, skull, and scalp
  - Failure of cephalic part of the neural tube to close
- Exencephaly
  - Skull and scalp are absent
  - Exteriorization of the abnormally formed brain
- Encephalocele
  - Herniation of cranial contents through defect in skull
- Iniencephaly
  - Triad of an occiptal bone defect, cervical dysraphism, and fixed retroflexion of the fetal head

Anencephaly

Cranial Defects
Neural Tube Defects

- Spina bifida
  - A cleft in the spinal column
    - Closed (the skin covering the defect is intact)
    - Open (not covered by skin)
- Spina bifida occulta
  - Failure of the dorsal portions of the vertebrae to fuse
  - Usually localized to the sacroiliac region
  - Covered by skin
  - Diagnosed by radiographs of spinal vertebrae

- Meningocele
  - Develops if more than one vertebrae involved in the defect
  - Only meninges of spinal cord herniates through opening
  - Not associated with hydrocephalus or neurologic defects
- Meningomyeleocele
  - Both meninges and spinal cord herniate through vertebral defect

Epidemiology

- Incidence: 1/1000 live birth
  - Incidence increased in Ireland and Wales
  - Decreased in Asia, Africa, and South America
- Recurrence rate: 4%
  - Whites > blacks (6:1)
  - F > M
Risk Factors

- Environment
- Genetics
- Socioeconomic status
- Maternal diabetes
- Maternal weight
- Maternal alcohol use
- History of NTD or a close relative
- Use valproic acid or carbamazepine
- Hispanic ethnicity

Folic Acid

Homocysteine levels in women who gave birth to children with neural tube defects significantly higher than those who gave birth to normal children.

- This would be expected to occur in pregnant women with low folate status
- Enzyme that metabolizes homocysteine to methionine, methionine synthase, uses 5-methyltetrahydrofolate, and vitamin B12, as cofactors

Folic Acid

- 50% -70% of defects prevented with folic acid daily before conception and through 1st trimester
- 1992 - to reduce number of cases of spina bifida and other NTDs, U.S. Public Health Service (USPHS) recommended that all women capable of becoming pregnant consume 400 µg of folic acid daily
  - Improve dietary habits
  - Fortify foods with folic acid
  - Use dietary supplements containing folic acid
Folic Acid vs Folate

- Folic acid
  - Synthetic form used in supplements and fortified foods
  - Body almost completely uses synthetic folic acid
- Folate
  - Found naturally in foods
  - Body only partially uses natural folate

Diagnosis and Detection

- Amniocentesis
  - AFP - indication of abnormal leakage
  - Acetylcholinesterase indicative of neural tissue
- Blood test
  - Maternal blood samples of AFP
- Ultrasonography
  - For locating back lesion vs. cranial signs

Sonographic Components

- Placenta
- Umbilical Cord
Placenta Accreta
- Placental lakes with vascular flow
- Prior uterine surgery
- Placenta previa
- Loss of hypoechoic uterine placental border

Two Vessel Umbilical Cord
- Variation of normal
- Associated with cardiac defects and renal abnormalities
- Associated with fetal growth restriction

Partial Molar Pregnancy (1)
- Triplet chromosomal complement
  - Most commonly
    - Two sets from paternal origin and one set from maternal origin
  - Associated fetus most always has anomalies
    - NTD
    - Short limbs
- Not biologically the same as hydatidiform (complete) molar pregnancy
Partial Molar Pregnancy (2)

- Focal villus swelling
- Focal trophoblastic hyperplasia
- Embryonic/fetal tissue
- Triploid karyotype
  - Ovum + 2 sperm
- Symptoms of missed abortion
- Persistent GTN - 4%

Moles

- Complete Hydatidiform Mole
  - Cyrognetics - Paternal DNA
  - 46XX (95%)
  - Diaphoric diploidy
- Partial Hydatidiform Mole
  - 46XX (5%)
  - Diaphoric diploidy

Comparisons

- Partial Mole
  - Diffuse villus swelling
  - Diffuse trophoblastic hyperplasia
  - No embryonic tissue
  - Diploid karyotype
    - 46, XX - all paternal
  - Excessive uterine enlargement and high bHCG
  - Persistent GTN - 20%
- Complete Mole
  - 46, XX - all paternal
Triploidy
- Cardiac defects
- Central nervous system anomalies
- Club feet
- Cystic hygroma
- Facial abnormalities - hypertelorism
- Intrauterine growth restriction
- Micrognathia
- Placenta
- Hydatidiform placental changes

Doppler Assessment

The Doppler Principle
Doppler Assessment

Umbilical Artery Doppler Waveforms

Statistics Review
Describing Data

- **Mean**
  - Average value
- **Median**
  - 50th percentile or quartile
  - Data point “in the middle”
  - ½ observations below, ½ observations above
- **Mode**
  - Most common value/result

Hypothesis Testing

- Estimating the real world based on a sample
  - \( H_0: \) Fail to reject the null hypothesis
  - \( H_1: \) Reject the null hypothesis

<table>
<thead>
<tr>
<th>Results of Statistical Test</th>
<th>The Real World</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( H_0 ) is true</td>
</tr>
<tr>
<td>Reject ( H_0 )</td>
<td>1-( \alpha )</td>
</tr>
<tr>
<td>Fail to Reject ( H_0 )</td>
<td>Type I ( \alpha )</td>
</tr>
</tbody>
</table>

P value

- Decision to reject or accept null hypothesis based on the "p" value
- Relative likelihood that observed exposure-disease relationship due to chance alone
- \( P < 0.05 \)
  - Less than a 5% likelihood that the observed results is due to chance alone
  - Less than a 5% chance that the decision to reject the null hypothesis is an error
  - Probability that a test statistic would be as extreme or more extreme than observed if the null hypothesis were true
Confidence Intervals

• Indication of the variability of a point estimate
  • Can refer to RR or OR
  • 95% often used
• If the 95% confidence limits DO NOT CROSS one, then the results ARE statistically significant
• If the 95% confidence limits CROSS one, then the results are NOT statistically significant

Sensitivity and Specificity

• $a$ - diseased individuals detected by the test (true positives)
• $b$ - nondiseased individuals positive by the test (false positives)
• $c$ - diseased individuals not detected by the test (false negatives)
• $d$ - nondiseased individuals negative by the test (true negatives)

<table>
<thead>
<tr>
<th>Screening Test Results</th>
<th>Disease Present</th>
<th>Disease Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>$a$</td>
<td>$b$</td>
<td>$a+b$</td>
</tr>
<tr>
<td>Negative</td>
<td>$c$</td>
<td>$d$</td>
<td>$c+d$</td>
</tr>
<tr>
<td>Total</td>
<td>$a+c$</td>
<td>$b+d$</td>
<td>$a+b+c+d$</td>
</tr>
</tbody>
</table>

• Sensitivity - $a/(a+c)$
  • Proportion of truly diseased persons in the screened population who are identified as diseased by the screening test
• Specificity - $d/(b+d)$
  • Proportion of truly nondiseased persons who are identified as nondiseased by the test
Predictive Value

- **Positive predictive value** - \( \frac{a}{a+b} \)
  - Probability that a person with a positive test has the condition which the test detects
  - Dependent upon prevalence of disease as well as sensitivity and specificity

- **Negative predictive value** - \( \frac{d}{c+d} \)
  - Probability that the patient will not have the disease when restricted to all patients who test negative
  - NPV = TN / (TN + FN)
  - The denominator for negative predictive value is the number of patients who test negative
  - **Positive Predictive Value** = True Positives/All Positives
  - **Negative Predictive Value** = True Negatives/All Negatives

Remember

- The sensitivity and specificity do not depend on the prevalence or pre-test probability of disease
- The predictive value varies with the pre-test probability of disease
Receiver Operator Curve

- Graphs tradeoff between sensitivity and specificity
  - Any increase in sensitivity results in a decrease in specificity
- Closer curve follows left-hand border and the top border of ROC space, the more accurate the test
- Closer curve comes to the 45-degree diagonal, the less accurate the test

Statistics - Summary

- **Sensitivity** - true positives
  - How well test identifies outcome of interest
- **Specificity** - true negatives
  - How well test identifies those without the outcome
- **Positive Predictive Value**
  - Chance that a positive test will result in the outcome
- **Negative Predictive Value**
  - Chance that a negative test will not result in the outcome
- **Likelihood Ratio (positive)**
  - How much more likely a finding will result in disease than one without the finding
  - \( \frac{TP}{TP+FN} \) / \( \frac{FP}{FP+TN} \)
  - \( \frac{TP}{TP+FN} \) - sensitivity
  - \( \frac{FP}{FP+TN} \) - specificity
- **Likelihood Ratio (negative)**
  - How much more likely absence of a finding results in absence of disease vs presence of a finding
  - \( \frac{FN}{TP+FN} \) / \( \frac{TN}{FP+TN} \)
  - \( \frac{FN}{TP+FN} \) - false negative rate
  - \( \frac{TN}{FP+TN} \) - true negative rate