Prenatal Genetic Screening and Testing: A Review of Current Strategies and Recommendations

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Objectives

- Review serum and ultrasound screening available for chromosomal defects and other fetal anomalies
- Understand differences between multiple available serum screening test protocols
- Review [diagnostic] tests for fetal aneuploidy
- Understand current standard of care regarding prenatal genetic screening and diagnosis
A case scenario...

- 36 year old G1P0 presents for first prenatal appointment
- 8 weeks and 2 days GA by certain LMP
- “I heard my baby could have Down Syndrome because I’m over 35. What should I do?”
## Maternal Age-Related Midtrimester Risks

<table>
<thead>
<tr>
<th>Age</th>
<th>Down Syndrome</th>
<th>All Aneuploidies</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>1/333</td>
<td>1/152</td>
</tr>
<tr>
<td>35</td>
<td>1/250</td>
<td>1/132</td>
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<tr>
<td>36</td>
<td>1/192</td>
<td>1/105</td>
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<tr>
<td>37</td>
<td>1/149</td>
<td>1/83</td>
</tr>
<tr>
<td>38</td>
<td>1/115</td>
<td>1/65</td>
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</tbody>
</table>

*Williams Obstetrics. 21st Ed. 2001.*
Historical Perspective

- Serum screening for Down Syndrome (DS) introduced in 1984
- Low maternal serum alpha-fetoprotein (AFP) associated with DS
- In 1990’s, human chorionic gonadotropin and unconjugated estriol were added → “triple screen”
Serum Screening Tests

- Serum values reported as “multiples of the median” (MoM)—i.e. a comparison to the median value of that particular marker in unaffected pregnancies
- Marker levels vary by gestational age
- Sensitivity is balanced with acceptable false positive rate
Serum Screening Tests

- Current serum markers include:
  - Serum alpha-fetoprotein (AFP)
  - Human chorionic gonadotropin (hCG)
  - Unconjugated serum estriol (uE3)
  - Inhibin-A
  - Free B-hCG
  - Pregnancy-Associated Plasma Protein-A (PAPP-A)
Triple vs. Quadruple Tests

- Standard midtrimester screening tool in the U.S. for 15 years = “multiple marker” or “triple screen” (serum AFP, hCG, and uE3) (Bahado-Singh R. Obst Gyn Clin N Am 2004)
- Performed between 15 and 22 weeks
- 1:270 risk = “positive” result (73% sensitivity; 9% false positive rate) (Benn PA. Clinica Chimica Acta 2002)
- Adding inhibin-A (“quadruple test”) gives 81% sensitivity and 5% false positive rate (Wald NJ. Health Technol Assess 2003)
First Trimester Screen

- Performed between 9 and 13 weeks
- Advantages and disadvantages
  - early reassurance or opportunity for diagnosis
  - no AFP, therefore does not eval NTD risk
- Free B-hCG and PAPP-A (67% sensitivity; 5% false positive rate used alone) (Wapner R. NEJM. 2003)
- Serum test optimal at 11 wks GA (Malone FD, Canick JA et al. NEJM 2005)
Nuchal Translucency

- Second component of the first trimester screen
- Ultrasound measurement of the thickness of the soft tissues at the back of the fetal neck
- Optimally done at 12-13 wks GA, but valid from 10 4/7 to 13 6/7 wks (ACOG Practice Bulletin No. 77)
- Measurement can be converted to a MoM
- Used with the serum markers, sensitivity is 79% and false positive rate is 5% (Wapner R. NEJM. 2003)
Nuchal Translucency

Souka et al. Ultrasound Obstet Gynecol 2001
Nuchal Translucency
First Trimester Screening

  - Spontaneous loss before 24 wks
  - Fetal demise
  - Low birth weight
  - Preterm birth

- No data yet supporting third trimester fetal surveillance
Integrated Screening Test

- Attempt to combine benefits of 1st and 2nd trimester screening
- 1st trimester screen performed, but results not disclosed
- 2nd trimester screen (quad test) obtained, total result calculated and disclosed
- Sensitivity 85% with false positive rate 1.2% (Wald N. J Med Screen. 2003)
- Sensitivity 94-96% with screen-positive rate 5% (Malone F, Canick J, et al NEJM 2005 = FASTER Trial)
- Disadvantage = no opportunity to consider CVS if first trimester screening shows high risk
Comparing Serum Tests

  - DS identification was the focus
  - 38,033 enrolled
  - GA 10w3d – 13w6d by U/S, singletons
  - 1st trimester screen obtained
  - Returned at 15 – 18 wks for quad test
  - If pos 1st or 2nd trimester screen, offered genetics counseling and amniocentesis
Comparing Screening Tests

- FASTER Trial false positive rates, at fixed 85% sensitivity
  - 1\textsuperscript{st} tri screen = 3.8-6.8% (increased b/w 11-13 wks)
  - Triple screen = 14%
  - Quadruple screen = 7.3%
  - Integrated screen = 0.6-1.2%
Comparing Serum Tests

- FASTER Trial: What about a stepwise sequential testing model?
  - 1st tri screen performed at 11 wks, results given. If pos, genetic counseling and chorionic villus sampling offered.
  - If neg, quad test performed at 15 wks
  - Ultimate result integrates findings of 1st and 2nd trimester into a final risk assessment
  - Sensitivity is 95%, with false positive rate 4.9%
  - At same 95% sensitivity, integrated screen gives false positive rate 4.0% (i.e., only slightly better than stepwise)
Comparing Serum Tests

- FASTER Trial: What about independent sequential screening?
- Quad test interpreted w/o reference to 1st trimester results
- For sensitivity of 94%, false positive rate is 11-17%
- False positive rate is essentially additive for the two tests
- This should not be used!
Contingent Model

- Another approach to sequential testing
- First trimester screen generates one of three possible results
- Cutoffs vary among institutions. One example:
  - “high” risk > 1:65
  - “intermediate” risk 1:65 to 1:1300
  - “low” risk < 1:1300
- High risk → offered CVS
- Low risk → no further testing
- Intermediate risk → offered second trimester test
- If both performed, both tests are used to calculate a final risk
### DS Detection at 5% Positive Screen Rate

(ACOG Practice Bulletin No. 77)

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Detection Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuchal Translucency (NT)</td>
<td>64-70</td>
</tr>
<tr>
<td>First Trimester Screen</td>
<td>82-87</td>
</tr>
<tr>
<td>Triple Screen</td>
<td>69</td>
</tr>
<tr>
<td>Quadruple Screen</td>
<td>81</td>
</tr>
<tr>
<td>Integrated Screen</td>
<td>94-96</td>
</tr>
<tr>
<td>Serum Integrated Screen (no NT)</td>
<td>85-88</td>
</tr>
<tr>
<td>Stepwise Sequential Screen</td>
<td>95</td>
</tr>
<tr>
<td>Contingent Sequential</td>
<td>88-94</td>
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</tbody>
</table>
Comparing Performance of Screening Tests

- Difference in spontaneous fetal loss rates between first and second trimester is important
- 9% of DS fetuses with cardiac activity in first trimester demise before the second trimester
- Studies that do not correct for these losses will overestimate the performance of first trimester risk assessment  
  (Reddy U, Wapner R. Clinical Obstet Gynecol 2007)
Conclusions in Comparing Screening Tests

- First trimester, second trimester, stepwise or contingent sequential and integrated screening are all acceptable.
- Choice will depend upon gestational age at presentation and patient preference.
  - Individual patient might accept first trimester screening, but decline full integrated/sequential screen due to unwillingness to terminate a second trimester pregnancy.
  - Quad test more expensive, therefore patients who present in second trimester may not have access to this test at certain sites (First Choice, First Nations, etc).
- Stepwise sequential screening allows earlier diagnosis, but gives slightly higher false positive rate than integrated screen.
- Cost-effectiveness is another matter!
“Let’s try getting up every night at 2:00 AM to feed the cat. If we enjoy doing that, then we can talk about having a baby.”
Ultrasound to Detect Aneuploidy and Other Anomalies

- Ultrasonographic “anatomic survey” at 18-20 weeks = important tool to dx NTD, other structural abnormalities
- May reveal markers for chromosome abnormalities
  - Major cardiac anomaly
  - Pyelectasis
  - Shortened femur or humerus
  - Echogenic bowel
  - Thickened nuchal fold
  - Echogenic intracardiac focus
  - Hypoplastic fifth digit
  - Sandal gap toe
Ultrasound Findings

Echogenic Bowel

ISUOG Educational Committee; Gianluigi Pilu, Kypros Nicolaides, Renato Ximenes, Philippe Jeanty
Ultrasound Findings

Echogenic Cardiac Focus

ISUOG Educational Committee; Gianluigi Pilu, Kypros Nicolaides, Renato Ximenes, Philippe Jeanty
Ultrasound Findings

Choroid Plexus Cyst

ISUOG Educational Committee; Gianluigi Pilu, Kypros Nicolaides, Renato Ximenes, Philippe Jeanty
Ultrasound to Detect Aneuploidy and Other Anomalies

- Age-related risk of DS can be multiplied by likelihood ratio of a given U/S marker
- Unreliability of obtaining markers makes this not practical in a low-risk population (Smith-Bindman R, Hosmer W, et al. JAMA 2001)
- Absence of all markers decreases DS risk by 50-60%
- Many anomalies are missed by U/S (Bahado-Singh RO, Oz U, et al. Semin Perinatol 2005)
  - 50-75% detection rate for DS in second trimester in high-risk population
  - For 100% detection rate, false positive rate increases to 21.9%
Ultrasound to Detect Aneuploidy and Other Anomalies

  - 93-100% detection if performed
    - at optimal GA of 18-21
    - by skilled sonographer
- Indicative findings
  - Clenched hands
  - Cardiac defect
  - Central nervous system abnormality
  - Choroid plexus cyst
- Isolated choroid plexus cyst not considered an indication for offering amniocentesis (if serum screen normal, anatomic survey not limited by body habitus, etc)
Ultrasound Screening for Neural Tube Defects

Normal

Spina Bifida

Meningomyelocele
Additional Ultrasound Screening Tools

- Nasal bone visualization
  - Absence confers higher risk of DS
  - FASTER Trial in United States failed to find it useful
  - Considered a more difficult skill to master than nuchal translucency measurement
Additional Ultrasound Screening Tools

- **Tricuspid Regurgitation**
  - Associated with aneuploidy and congenital heart disease

- **Ductus Venosus**
  - Originates from umbilical vein and empties into inferior vena cava
  - Directs highly oxygenated blood toward fetal heart
  - Reversed flow in the DV associated with aneuploidy and congenital heart disease \[\text{(Nicolaides KH, Spencer K. Ultrasound Obstet Gynecol. 2005)}\]
    - 82.4% of DS fetuses
    - 5.0% of euploid fetuses

- Both are technically difficult measurements and not yet standard of care until better quality control is achieved
“It’s a new medical technology. Instead of crying, we can program your choice of 200 fun ring tones!”
Diagnostic Tests

- Historically, amniocentesis has been offered
  - ≥35 at time of delivery
  - Positive screening test
  - History of prior affected pregnancy
- Performed at 15-20 weeks
- Risk of pregnancy loss after amnio = 1:200 (hence triple screen “positive” at 1:270)
Diagnostic Testing

- Chorionic villous sampling indications similar to amnio
- Performed at 10-13 weeks
- Transcervical or transabdominal
- Pregnancy loss rate ~1:100
- Only amnio and CVS are diagnostic (i.e. retrieve chromosomal material)
Diagnostic Testing

- Current ACOG recommendation: “All women, regardless of age, should have the option of invasive testing.” (ACOG Practice Bulletin No. 77)
- Women’s decisions based on multiple factors (ACOG Practice Bulletin No. 77)
  - Individual risk of chromosomal abnormality
  - Risk of pregnancy loss from invasive procedure
  - Consequences of having an affected child
- Studies show that women weigh these factors differently, and that their perception of their risk, not their actual risk, most influences their decision
All women should be offered aneuploidy screening before 20 wks gestation…”

“It is not practical to have patients choose from among the large array of screening strategies…”

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

“Info. about the detection and false-positive rates, advantages, disadvantages, and limitations, as well as the risks and benefits of diagnostic procedures, should be available to patients so that they can make informed decisions.”
ACOG Recommendations

“It is preferable to provide patients with their numerical risk determined by the screening test, rather than a positive versus negative screening result using an arbitrary cutoff.”
ACOG Recommendations: Some Caveats

- If NT measurement not available or cannot be obtained in a particular patient
  - Offer *serum* integrated screening to patients who present early
  - Offer second trimester screening to those who present later
ACOG Recommendations: Some Caveats

- Patients who have only first trimester screens or who had a normal CVS should be offered neural tube defect screening
  - Second trimester serum AFP
  - Ultrasonography

- If nuchal translucency $\geq 3.5$ mm (despite negative aneuploidy screen or normal fetal chromosomes)
  - targeted U/S exam, fetal echo, or both should be offered
  - increased risk of non-chromosomal anomalies
    - congenital heart defects
    - abdominal wall defects
    - diaphragmatic hernias
    - genetic syndromes
Back to the case scenario...
How should we counsel our patient?

- 1st step = discuss pro’s and con’s of screening in general, and limitations
- Review availability of diagnostic tests
- Patient need not know what she would do with positive screen (CVS, amnio, continue vs. terminate pregnancy if confirmed by diagnostic test)
Case scenario...

- Combined 1\textsuperscript{st} and 2\textsuperscript{nd} trimester testing provides the best sensitivity and lowest false-positive rate, if screening is desired.
- Independent sequential 1\textsuperscript{st} and 2\textsuperscript{nd} trimester screens not recommended.
- Integrated (delayed results) or sequential tests are acceptable, as are individual first or second trimester tests.
- If CVS or amnio desired by pt anyway, no serum screening indicated.
- Ultrasound in second trimester can reveal anomalies not evaluated with serum screening.
How do women feel about prenatal genetic screening/testing?

- Cleveland Clinic qualitative study using focus groups (Farrell R., Dolgin N., et al. Genetics in Medicine 2011)
- Outpatient obstetric clinics as patient source
- 18-45 years of age, N= 46
- Current or past pregnancy
- English-speakers
- Assigned to group
  - Pregnant for first time
  - Pregnant for second or greater time
  - Prior but not current pregnancy
How do women feel about prenatal genetic screening/testing?

- All watched 5 minute informational video re. first tri aneuploidy screening and testing options
- Discussion moderated w/ open-ended questions
How do women feel about prenatal genetic screening/testing?

- Participants reported first tri as physically and emotionally tumultuous
- Range of emotions related to whether pregnancy was planned or not
- Those w/ infertility hx reported anxiety, disbelief, emotional distancing from the pregnancy
How do women feel about prenatal genetic screening/testing?

- Most unaware that all women are at risk of having fetuses with genetic abnormalities.
- Though they could articulate risks and benefits of screens/tests, they expressed internal conflict re. the balance.
- Most thought earlier testing was better, to allow earlier termination or longer period of reassurance.
How do women feel about prenatal genetic screening/testing?

- Also prominent: difficulty getting in to see provider in first tri time frame, overwhelming amount of info presented at first visit
- Current research underway evaluating decision aides re. prenatal genetic testing options
- UNM CNMs have developed a patient education tool regarding genetic testing
What about pre-conception genetic evaluation?

- **Family Hx** (Wilson R et al. J Obstet Gynaecol Can. 2011)
  - Three generation pedigree
  - Genetic disease: muscular dystrophy, hemophilia, cystic fibrosis, fragile X, congenital heart disease, phenylketonuria, dwarfism, sickle cell, Tay-Sachs, deafness, spinal muscular atrophy
  - Multifactorial congenital malformations: spina bifida, anencephaly, cleft lip and palate, hypospadias, congenital heart disease
What about pre-conception genetic evaluation?

- Risks related to ethnic origins: sickle cell, Tay-Sachs, thalassemias
- Consanguinity: “Are you and your partner related to each other in any way other than by marriage?”
- Couples w/ 3 or more SABs: 5.5% incidence of one partner having balanced translocation
What about pre-conception genetic evaluation?

- **Age-related risks**
  - Maternal: aneuploidies
  - Paternal: new dominant single gene mutations (achondroplasia, neurofibromatosis, Marfan syndrome, osteogenesis imperfecta, other syndromes)
  - Maternal grandfather (of male fetus) via X-linked: hemophilias A & B (factors VIII and IX), Duchenne MD, Hunter syndrome, X-linked agammaglobulinemia, retinitis pigmentosa
Testing Sites

- UNM
  - FPC for dating U/S at ≤ 12 wks GA
  - Genetics Consultation at Women’s Imaging
    - Nuchal Translucency at 11 3/7 – 13 5/7 wks GA
    - Serum markers drawn in lab after U/S appt

- New Mexico Sonographics
  - Nuchal Translucency at 11 0/7 –13 6/7 wks
  - Clinician must arrange for serum markers to be drawn, and report the NT to the lab calculating the result

- Private perinatologists: Center for Perinatal Development, Pinon Perinatal, etc.