Carrier Screening in the Pre-gestational / New Obstetrical Patient

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Presenter Disclosure

Dr. **Donnenfeld** has no Conflict of Interest to disclose

Dr. **Donnenfeld** has Financial disclosure:
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Dr. **Donnenfeld** has no Off-Label disclosures.
Learning Objectives

To understand the optimal approach to screening pre-gestational and new obstetrical patients to determine their risk to have a child with Spinal Muscular Atrophy.

To review the clinical spectrum of findings in children with Cystic Fibrosis.

To identify features present in permutation carriers of Fragile X syndrome.

Pre-gestational and new obstetric patients are capable of self-regulation, self-healing, and health maintenance including determining their risk to have a child with a serious genetic disorder.

Permutation carriers of Fragile X syndrome may develop behavioral, neurologic, and reproductive problems that impact them through a combination of body, mind and spirit.
The Evolution of Prenatal Screening

- Down Syndrome
- Neural Tube Defects
- Trisomy 18
- Cystic Fibrosis
- Sickle Cell Anemia
- Alpha and Beta Thalassemia
- Ashkenazi Jewish Genetic Diseases
- Spinal muscular atrophy
- Fragile X syndrome
- Noninvasive prenatal screening
- Expanded Carrier Screening

To Infinity and Beyond?
1. Cystic Fibrosis
Facts about Cystic Fibrosis

- CF is the most common serious autosomal recessive disorder in Caucasians
- CF occurs in approximately 1:2,500 live Caucasian births in the U.S.
- Median survival of a CF patient is approximately 31 years
- One in 31 Americans (more than 10 million) are carriers
- The carrier frequency and incidence varies among ethnic groups
- CF is the result of a basic defect in the (CFTR) gene
Survival of Patients with CF

Median Survival Age (Year)


Cumulative Survival

Age (Yrs)

0 6 12 18 24 30

Normal Births

Meconium Ileus
Autosomal Recessive Inheritance

NN
NAB
ABN
ABAB

Unaffected non-carrier
Unaffected carriers

75% Chance Unaffected
25% Chance Affected
## CF Detection Rate by Ethnicity

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Carrier Freq</th>
<th>23 mutations</th>
<th>97 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>1/29</td>
<td>80%</td>
<td>93%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1/46</td>
<td>57%</td>
<td>78%</td>
</tr>
<tr>
<td>African</td>
<td>1/60</td>
<td>75%</td>
<td>81%</td>
</tr>
<tr>
<td>Asian</td>
<td>1/90</td>
<td>30%</td>
<td>38%</td>
</tr>
<tr>
<td>Ash Jewish</td>
<td>1/29</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>75%</strong></td>
<td><strong>84%</strong></td>
</tr>
</tbody>
</table>
Who should be Offered Routine CF Prenatal Carrier Testing?

All prenatal patients

Testing should be offered early enough in pregnancy (up to 20 weeks) to allow for possible fetal testing

A negative screening test does not eliminate the possibility that an individual is a carrier of the CF gene
2. Spinal Muscular Atrophy
Carrier screening for spinal muscular atrophy

Thomas W. Prior, PhD, for the Professional Practice and Guidelines Committee

Key Words: spinal muscular atrophy, carrier screening, genetic testing, population screening
Screening for Spinal Muscular Atrophy (SMA)

- SMA fits the criteria for population-based screening
- Testing should be offered to all couples, regardless of race/ethnicity
- Identified carrier couples should be referred for genetic counseling
American Congress of Obstetrics and Gynecology

Committee Opinion 691, March 2017
Carrier Screening for Genetic Conditions
Spinal Muscular Atrophy (SMA)

Screening for Spinal Muscular Atrophy should be offered to all women who are considering pregnancy or are currently pregnant.
Spinal Muscular Atrophy (SMA)

- Most common inherited cause of infant mortality
- Genetic disease characterized by the progressive degeneration of the lower motor neurons
- Autosomal recessive inheritance
  - 2nd most common autosomal recessive lethal condition in the U.S. after cystic fibrosis
  - ~1 in 41 carrier frequency
- Affects all racial and ethnic groups
  - 1 in 6,000-10,000 live births

## Spectrum of Clinical Manifestations

<table>
<thead>
<tr>
<th>SMA Type</th>
<th>Age of Onset</th>
<th>Typical Life Span</th>
<th>Formerly Called</th>
<th>Clinical Characteristics/Milestones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenat.</td>
<td>Prenat.</td>
<td>&lt;6 months</td>
<td>Congenital axonal neuropathy, arthrogryposis multiplex congenita</td>
<td>No milestones achieved. Severe weakness. Early respiratory failure.</td>
</tr>
<tr>
<td>II</td>
<td>Birth-6months</td>
<td>25 years</td>
<td>Independent sitting, with loss of this ability by the mid-teens. Early respiratory failure.</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>After 12mo.</td>
<td>Normal</td>
<td>Kugelberg-Welander</td>
<td>Ambulation, with loss of this ability as disease progresses.</td>
</tr>
<tr>
<td>IV</td>
<td>Adult/Normal</td>
<td>Normal</td>
<td>Ambulation, with loss of this ability as disease progresses.</td>
<td></td>
</tr>
</tbody>
</table>

**Type I is the most common (60-70%) and the most severe.**
An Example of SMA Type 1 Disease Progression

- Typically a healthy, “normal” child at birth
- Initial concern at a 2 or 4 month check-up when child cannot support his/her head and/or exhibits “floppiness”
- At 4 to 6 months, the child will lose the ability to swallow
  - Family will have to decide whether or not to insert a feeding tube or to suction child’s nasal secretions and saliva
- Typically at 6 to 9 months, the child will have difficulty with respiratory infections and breathing
  - Family will have to decide about respiratory intervention
  - If respiratory intervention is not used, most children will die from respiratory failure by age 2
  - If respiratory intervention is used, the child may live longer, but with significant morbidity
Genetics of SMA

- Caused by a deletion or mutation in the survival motor neuron 1 (SMN1) gene
- Discovered in 1995
- Located on chromosome 5q11-q13
SMA Carrier Testing

- Determine $SMN1$ copy number
  - Quantitative real-time PCR
- $SMN1$ copy number analysis detects $\sim94\%$ of carriers
SMA Carrier Result Interpretation

1 copy $SMN1 = \text{carrier}$

2 copies $SMN1 = \text{reduced carrier risk}$

The probability of being an SMA carrier is reduced from 1/41 to 1/648 if an unaffected individual without a family history of SMA is found to have 2 copies of $SMN1$

3. Fragile X Syndrome
Fragile X Syndrome

Affected individuals present with

- variable mental impairment ranging from severe MR to DD and autism
- large or prominent ears, long face and prominent chin
- characteristic speech, language and behavioral features
## Fragile X Syndrome

The leading inherited cause of mental retardation

### Carrier Frequency
~1 in 260 females

### Incidence
1 in 4,000 males, 1 in 8,000 females

### Inheritance
X-linked

### Clinical Characteristics
- Mild learning disabilities to severe mental retardation
- Autism and hyperactivity
- Almost all males with full mutations are mentally retarded, ranging from developmental delay to severe mental retardation
- ~50% of females with a full mutation have IQs in the borderline or mentally retarded range; of the remaining 50%, as many as half have learning disabilities

### Typical Lifespan
Normal

### Detection Rate
~99%

Approximately 1/3 of all children diagnosed with fragile X syndrome also have autism and hyperactivity.

50% of families have a 2\textsuperscript{nd} child before they find out their 1\textsuperscript{st} child has fragile X. \textsuperscript{4}
FMR-1

- FMR-1 gene located on the X chromosome (1991)
- Gene product is FMR-1 protein
- FMR-1 is absent in full mutation males
- Loss of function interferes with normal brain development
Fragile X Syndrome: Molecular Basis

- Caused by additional copies of a CGG (triplet) repeat
- Unstable and can change in size when passed from parent to offspring
- Rare exceptions: De novo deletions and point mutations
Fragile X Testing

- Gold standard: Southern blot and PCR determine repeat size and methylation status of the allele
- Four categories for diagnosis:

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of CGG Repeats</th>
<th>Clinical Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>&lt;45</td>
<td>Normal <em>FMR1</em> gene.</td>
</tr>
<tr>
<td>Intermediate</td>
<td>45-54</td>
<td>Not at risk to have fragile X affected offspring.</td>
</tr>
<tr>
<td>Premutation</td>
<td>55-200</td>
<td>Intellectually normal but at risk for fragile X-associated POF and FXTAS and for offspring with Fragile X syndrome</td>
</tr>
<tr>
<td>Full mutation</td>
<td>&gt;200</td>
<td>&gt;99% males and ~50% of females mentally impaired.</td>
</tr>
</tbody>
</table>
Fragile X Carrier Screening

- Indicated if there is a family history of mental retardation or a family hx of Fragile X syndrome
- Many obstetric and perinatology practices choose to offer fragile X carrier testing to all women because
  - fragile X is the most frequent inherited cause of mental retardation
  - fragile X syndrome is found among all ethnic groups
  - fragile X syndrome can occur in families with no history of mental retardation
4. Circulating Cell-Free Fetal (ccff) DNA: Noninvasive Prenatal Testing (NIPT)

- The source of ccff DNA is thought to be from placental and fetal-derived cells
- Recent studies indicate 5-20% of DNA in maternal blood is actually fetal in origin

Lo, et al., 1997 Lancet; Finning et al., 2002 Transfusion; Bianchi, Placenta 2004; Ding et al., 2004 PNAS; Gautier et al., 2005 American Journal of Obstetrics and Gynecology.
cfDNA in Maternal Blood

- Both cell-free **fetal** and cell-free **maternal** DNA circulate in maternal plasma. Fetal DNA comprises about 10% of the total circulating cell-free DNA in maternal plasma.

- Cell-free fetal and maternal DNA circulate in maternal plasma as short pieces (50-300 base pairs) and represent fragments from the entire genome.
The total number of ccf-fetal fragments plus ccf-maternal fragments of any one chromosome is identified. This is consistent from sample to sample, and patient to patient.

Sequencing tells you which chromosome the combined maternal and fetal fragments come from.
CCF DNA Testing From Maternal Blood (Massively Parallel DNA Sequencing)

Sequencing tells you which chromosome the circulating cell free fragment comes from.

1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  X  Y

TCCGCCAGCCGAGCTGAGGAGGCTGGAAATTGCTGAT chr21
GGCCCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
GACACGTGAGCTCGCCACACAGGAGACATGCTGATTCATGCTGAT chr14
GGCCCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
ACAGTGTGAGGAGCCATCGCTGAGCGCTGACTGACTT chr21
GGCCGCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
GGCCGCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
TCCGCCAGCCGAGCTGAGGAGGCTGGAAATTGCTGAT chr21
GACACGTGAGCTCGCCACACAGGAGACATGCTGATTCATGCTGAT chr14
GGCCCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
ACAGTGTGAGGAGCCATCGCTGAGCGCTGACTGACTT chr21
GGCCGCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
GGCCGCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
GACACGTGAGCTCGCCACACAGGAGACATGCTGATTCATGCTGAT chr14
GGCCCTGAGAGACACGAGCTCAAATCCACTGATTCATGCTGAT chr10
Chromosome Dosage Analysis for Trisomy 21

- Measure the small DNA fragments derived from chromosome 21 in maternal blood.
- This measurement includes both maternal and fetal chromosome 21 fragments.
- If the amount exceeds a critical threshold relative to expected amounts from normal reference samples, it indicates an excess number of chromosome 21’s (i.e. trisomy 21).
- Analysis can be done anytime after 10 weeks gestation.
- Turn around time (currently) approximately 7 days.
Principles of Fetal Trisomy 21 Detection Using DNA Sequencing

*DNA analysis does not differentiate which fragments come from the mother and which from the fetus.*

The quantitative over-representation of Trisomy 21 fragments in an affected pregnancy is significant and can be measured with high precision.

Unaffected Fetus  

Fetus with Trisomy 21
Noninvasive Prenatal Testing (NIPT)
Circulating Cell-free Fetal DNA (CfDNA)

- Trisomy 21
- Trisomy 18
- Trisomy 13
- Sex chromosome disorders (XXX, XXY, XYY, 45,X)
- Microdeletions
CCF Fetal DNA vs Standard Prenatal Aneuploidy Screening

- 1914 women, mostly low risk, mean age 29.6 years, screened for trisomy 18 and 21
- False + rate for NIPT 0.5%
- False + rate for standard screening 4.2%
- PPV for NIPT 46%, PPV for std screen 4%
- NIPT performed 10 x better than standard screening, significantly lower false +’s and significantly better PPV

Bianchi et al, NEJM2014
NEXT Study (Ariosa Lab) Prospective Study

- 15,086 women, most young, low risk
- 36/36 DS cases detected by NIPT
- 28/36 DS (80%) cases detected by 1st TM screen
- False + rate 1st TM screen; 5.4%
- False + rate NIPT; 0.06%
- PPV 1st TM screen: 3%; NIPT 80%

Norton et al NEJM, 2015
Implications

“In every measure, the cell free DNA test outperformed standard screening. It demonstrates that this is not a test that should be limited to high risk women;”

Ron Wapner, PI NEXT study
ACMG abstract 2014
<table>
<thead>
<tr>
<th>Sequential</th>
<th>NIPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 blood tests &amp; an US</td>
<td>One blood test</td>
</tr>
<tr>
<td>11-14 wks &amp; 15-20 wks</td>
<td>Anytime after 10 wks</td>
</tr>
<tr>
<td>FPR 4%</td>
<td>FPR 0.1% (40 x better)</td>
</tr>
<tr>
<td>Det’n rate 90%</td>
<td>Det’n rate 99%</td>
</tr>
<tr>
<td>Tri 18 and 21</td>
<td>Tri 13, 18, and 21, sex chromosomes</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Screens for NTD’s</td>
<td>Does not screen for NTD’s</td>
</tr>
<tr>
<td>Contraindication: bleeding</td>
<td>Can be done if bleeding</td>
</tr>
<tr>
<td>Donor egg: Yes</td>
<td>Donor egg: Yes, most labs</td>
</tr>
<tr>
<td>Vanishing twins: no</td>
<td>Vanishing twins: no</td>
</tr>
<tr>
<td>Twins: yes</td>
<td>Twins: Most labs</td>
</tr>
</tbody>
</table>
Why is biochemical screening for Down Syndrome like Woody from Toy Story? They’re both old, reliable, past their prime?, and there’s something better
Noninvasive Prenatal Testing (NIPT)
Circulating cell free fetal DNA (cfDNA)
Microdeletion NIPT testing

22q11 (DiGeorge syndrome)
1p36 deletion (severe ID)
5p- (Cri du Chat)
15q11 deletion (Angelman and Prader Willi syndromes)
11q deletion (Jacobsen synd)
8q deletion (Langer-Gideon S)
Examples of conditions that can be identified by Microdeletion NIPT

**Williams syndrome**  
(Chromosome 7q11.23)

[Image: http://medgen.genetics.utah.edu/photographs/diseases/high/williams_syndrome_original1.gif]

**Wolf-Hirschhorn**  
(Chromosome 4p16.3)

[Image: From: Atlas of Genetic Diagnosis and Counseling (2002)]
Examples of conditions that can be identified by Microdeletion NIPT

- **Prader-Willi syndrome**
  (Paternal Chromosome 15q11-q13) deletion

- **Angelman syndrome**
  (Maternal Chromosome 15q11-q13) deletion

From http://www.specialchild.com/archives/poster-child002.jpg
Why screen for microdeletions?

The combined at-birth incidence of these 5 microdeletion syndromes is approximately 1 in 1,000, similar to the overall rate observed for Down syndrome.

The most common microdeletion, 22q11.2 deletion, is more common than trisomies 13 and 18 combined, and is more common than cystic fibrosis.

The risk for microdeletions is independent of maternal age, unlike whole chromosome aneuploidies like Trisomy 21 that are more prevalent in women of advanced maternal age. For pregnant women under the age of 29, this means they are more likely to have a fetal microdeletion than Down syndrome.
Genome-wide analysis by NIPT

GENOME-wide testing is capable of identifying > 95% of deletions or duplications ≥ 7 Mb

Will undoubtedly detect numerous variants of unknown significance (VOUS) that will complicate genetic counseling
5. Expanded Carrier Screening

The purpose of Expanded Carrier Screening is to serve as a general screening option for assessment of a multitude of genetic disorders, offered prior to conception or early in pregnancy.
Expanded Carrier Screening

Genotyping array targeting a panel of ~450 common mutations in >100 genes associated with ~90 autosomal recessive Mendelian disorders.

Advantages

- Low cost, Able to screen for many disorders/mutations in one test
- Requires just one tube of blood
- Appropriate across broad US population

Limitation of array-based genotyping typically detects only the common mutations for each disorder, not the entire genetic sequence

- Testing for some disorders is not possible
Essential criteria for carrier screening

– The disorder is a clinically severe health problem
– The disorder has a high carrier frequency in the population to be screened
– Reliable test exists with high specificity, sensitivity and predictive value
– Results are understandable, and access to genetic counseling is available
– There is no stigmatization or discrimination associated with being a carrier
– Testing is voluntary and informed consent is obtained
– Prenatal diagnosis is available
– The benefits of testing outweigh the harms
Ethnic-specific, panethnic, and expanded carrier screening are acceptable strategies for pre-pregnancy and prenatal carrier screening. Because all of these are acceptable strategies, each obstetrician–gynecologist or other health care provider or practice should establish a standard approach that is consistently offered to and discussed with each patient, ideally before pregnancy. Carrier screening will not identify all individuals who are at risk of the screened conditions. Patients should be counseled regarding the residual risk with any test result.
Expanded carrier screening

Joint statement of the Perinatal Quality Foundation, ACMG, ACOG, NSGC, and SMFM

“Condition-directed carrier screening” is limited because of “inaccurate knowledge of ancestry in our increasingly multiethnic society” and “recognition that genetic conditions do not occur solely in specific ethnic groups”

Positive results

In one series of 27,000 patients, 5223 individuals were positive for one or more diseases (19%)

- 4540 positive for one disease (17%)
- 604 positive for two diseases (2%)
- 71 positive for three diseases (0.3%)
- 8 positive for four diseases (0.03%)

Data on file (February 2016)
What does a negative result mean?

A negative result will decrease, but not eliminate, the risk of any individual to be a carrier for any specific disease in the test panel.

What does a positive result mean?

Test the partner

If the partner is negative, it reduces, but does not eliminate the risk of having an affected fetus.

If the partner is positive, there is a 25% risk for the fetus to be affected.

Sequencing of the partner?
Expanded Carrier Screening

Disorder List

Adenosine Deaminase Deficiency
Alpha-Mannosidosis
Andermann Syndrome
Argininosuccinic Aciduria
Aspartylglucosaminuria
Ataxia-Telangiectasia
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)
Bardet-Biedl Syndrome, BBS1-related
Bardet-Biedl Syndrome, BBS10-related
Beta Hemoglobinopathies, Other (C, D, E, O)
Beta-Thalassemia
Bloom Syndrome
Canavan Disease
Cartilage-Hair Hypoplasia
Citrullinemia Type I
Cobalamin C Disease (Methyalmalonic Aciduria with Homocystinuria)
Congenital disorders of Glycosylation, Type 1a
Cystic Fibrosis
Cystinosis
D-Bifunctional Protein Deficiency
Dihydrolipoamide Dehydrogenase Deficiency
Dihydropyrimidine Dehydrogenase Deficiency
Ethylmalonic Encephalopathy
Familial Dysautonomia
Familial Hyperinsulinism, ABCC8-related
Familial Mediterranean Fever
Fanconi Anemia Group C
Galactosemia, GAL-T related
Gaucher Disease
Glutaric Acidemia Type I
Glutathione Synthetase Deficiency
Glycine Encephalopathy, GLDC-related
Glycogen Storage Disease Type 1a
Glycogen storage disease Type Ib
Glycogen storage disease Type IIIa and IIIb
GRACILE Syndrome
Hereditary Fructose Intolerance
HMG-CoA Lyase Deficiency
Holocarboxylase Synthetase Deficiency
Homocystinuria, CBS-related
Joubert Syndrome 2
Junctional Epidermolysis Bullosa, LAMA3-related
Junctional Epidermolysis Bullosa, LAMB3-related
Junctional Epidermolysis Bullosa, LAMC2-related
Krabbe disease
Leigh syndrome, French-Canadian Type
Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)
Maple Syrup Urine Disease Type 1a
Maple Syrup Urine disease Type 1b
Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)
Metachromatic Leukodystrophy, ARSA-related
Methylmalonic Acidemia, MMAA-related
Methylmalonic Acidemia, MMA8-related
Methylmalonic Acidemia, MUT-related
Mucolipidosis Type IV
Mucopolysaccharidosis Type I
Nemaline Myopathy, NEB-related
Nephotic Syndrome, NPHS1-related
Nephotic syndrome, NPHS2-related
Neuronal Ceroid-Lipofuscinosis, CLN3-related
Neuronal Ceroid-Lipofuscinosis, CLN5-related
Neuronal Ceroid-Lipofuscinosis, CLN8-related
Neuronal Ceroid-Lipofuscinosis, PPT1-related
Neuronal Ceroid-Lipofuscinosis, TPP1-related
Niemann-Pick Type A
Niemann-Pick Type B
Niemann-Pick Type C, NPC1-related
Niemann-Pick Type C, NPC2-related
Nijmegen Breakage Syndrome
Phenylalanine Hydroxylase Deficiency (includes PKU)
Polycystic Kidney Disease, Autosomal Recessive
Pompe Disease
Primary Hyperoxaluria Type 1
Primary Hyperoxaluria Type 2
Propionic Acidemia, PCCA-related
Propionic Acidemia, PCCB-related
Rhizomelic Chondrodysplasia Punctata Type 1
Salla Disease
Sandhoff Disease
Sickle Cell Disease
Sjogren-Larsson Syndrome
Smith-lemli-opitz Syndrome
Sulfate Transporter-Related Osteochondrodysplasias:
  Achondrogenesis Type 1B
  Atelosteogenesis Type 2
  Diastrophic Dysplasia
  Recessive Multiple Epiphyseal Dysplasia
Tay-Sachs Disease
Tyrosinemia Type 1
Usher Syndrome Type IF
Usher Syndrome Type III
Walker-Warburg Syndrome, FKTN-related
Wilson Disease
Zellweger Syndrome Spectrum, PEX1-related:
  Zellweger Syndrome
  Neonatal Adrenoleukodystrophy
  Infantile Refsum Disease
Oh, crap! Was that TODAY?