Universal Carrier Screening: Promise and Perils

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Disclosures

• Nothing to disclose.
Current controversies

Objectives, overview:

• Current, esp. conflicting and/or confusing, practice recommendations
  ▪ Review current screening recommendations
  ▪ Fragile X Screening
  ▪ Carrier screening for Spinal Muscular Atrophy (SMA)

• Universal Carrier Screening
  ▪ Pros and cons
Practice Guidelines

- ACOG and ACMGG (the American College of Medical Genetics and Genomics) both provide recommendations for prenatal screening for specific genetic diseases
- In several situations, the guidelines are different
- This is confusing and potential medico-legally problematic
Genetic Diseases are Not as Rare as we Think!

2-3% of newborns have a congenital disease or malformation

These result in:

- More than 20% of infant mortality
- 30% of ICN admissions
Genetic Advances

Availability of prenatal testing for genetic disorders is constantly increasing.

Increasing numbers of tests available for:
- Single gene defects
- Chromosomal abnormalities
- Structural birth defects
Increase in available genetic tests

Data source: GENETests database (2010/www.genetests.org)
Carrier screening

Goal is to identify asymptomatic carriers with no family history of disease

As more tests become available, questions arise:

- Which should be offered?
- Who should decide?
- Who should pay?
- What is our medico-legal responsibility?
Heterozygote (Carrier) Screening

Most are autosomal recessive disorders

- Carriers typically asymptomatic
- Usually no family history
- Affect males and females equally
- Risk for carrier parents to have an affected child is 1/4 for each pregnancy
Ethnicity-Based Screening

- Frequency of many disorders varies among ethnic groups
- Effectiveness of screening also varies by ethnicity
  - Different populations have different mutations that cause the disorders
  - Testing usually targets the commonly affected groups, less effective in non-target populations
<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Common Genetic Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi Jews</td>
<td>Tay Sachs disease, Canavan disease, cystic fibrosis, familial dysautonomia</td>
</tr>
<tr>
<td>Louisiana Cajun, Fr Canadian</td>
<td>Tay Sachs disease</td>
</tr>
<tr>
<td>Caucasians</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Africans, African Americans</td>
<td>Sickle cell anemia, beta thalassemia</td>
</tr>
<tr>
<td>Southeast Asians</td>
<td>Alpha thalassemia</td>
</tr>
<tr>
<td>Mediterraneans</td>
<td>Beta thalassemia</td>
</tr>
</tbody>
</table>
Ethnicity Based Screening

- May present barriers by requiring knowledge of who to screen for which disorders
- Perpetuates categorizing of patients by race and ethnicity
  - Can be seen as stigmatizing
- Less robust with increasing multiculturalism
  - Less clear how to assign patients to a single ethnic or racial group in modern society
Heterozygote screening

- Tay Sachs disease 1971
- Hemoglobinopathies 1970’s
- Canavan disease 1998
- Cystic fibrosis 2001
- Familial dysautonomia 2004
- Spinal muscular atrophy 2008*
- Jewish disease panel 2008*
- Fragile X
- Universal screening

*ACMG only
Tay Sachs Disease

- TSD is a lysosomal storage disease caused by hexosaminidase A (hex A) deficiency
- Resultant accumulation of GM2 gangliosides results in progressive neuro-degeneration
- Death in early childhood
- There is no treatment or cure
Hex A Activity in Tay Sachs Disease
Ashkenazi Jewish Screening

- Screening for Tay Sachs disease was one of the first public health genetic programs.

- Carrier screening has resulted in a dramatic decrease in the frequency of TSD in this group.
Even Tay Sachs screening gets complicated, however…
Enzyme assay vs DNA?

- Initially screening involved enzyme assay for Hexosaminidase A activity
- More recently, a DNA test was developed
- Both have good sensitivities and specificities, although neither is perfect
  - DNA testing preferable in most cases
  - Enzyme screening is better for non-Ashkenazi Jewish individuals
- In complex cases, a combination of tests may be required
Who to Screen?

Groups at increased risk:
- Ashkenazi Jewish 1/27
- Louisiana Cajun 1/27
- French Canadian 1/27-73
- Irish Americans 1/50
- Pennsylvania Dutch ??

Groups not at increased risk
- Non-Jewish, Sephardic 1/250
Ashkenazi Jewish Screening

• Screening is available for several other disorders associated with AJ ancestry
  ▪ ACOG recommends screening for TSD, Canavan disease, cystic fibrosis, familial dysautonomia
  ▪ All are relatively common (1/40) and severe
• Testing also available for Bloom syndrome, Fanconi anemia, Gaucher disease, mucolipidosis IV, Niemann-Pick disease
  ▪ Most severe, untreatable and relatively rare
  ▪ 1/5 Ashkenazi Jews carries one of these nine disorders
<table>
<thead>
<tr>
<th>Disease</th>
<th>Carrier Frequency</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanconi Anemia Group C</td>
<td>1/89</td>
<td>99%</td>
</tr>
<tr>
<td>Niemann-Pick Type A</td>
<td>1/90</td>
<td>95%</td>
</tr>
<tr>
<td>Mucolipidosis IV</td>
<td>1/127</td>
<td>95%</td>
</tr>
<tr>
<td>Bloom Syndrome</td>
<td>1/100</td>
<td>95-97%</td>
</tr>
<tr>
<td>Gaucher Disease</td>
<td>1/15</td>
<td>95%</td>
</tr>
</tbody>
</table>
Ashkenazi Jewish Screening

• “Panels” of 8-10 disorders are available
• Most have carrier frequency of ~1/90
• Panels include Gaucher disease, which is common, has a variable phenotype, is often asymptomatic, and effective treatment exists
• Connexin 26 (deafness) also available for genetic testing

➢ Availability of tests raises ethical, medico-legal, and practical difficulties
Practice Guidelines: Ashkenazi Jewish Diseases (AJD)

ACOG + ACMG agree
- CF, Canavan, FD, TSD recommended for all women who are pregnant or considering pregnancy
- If only one partner is AJ, test that partner first. If positive, test non-AJ partner with enzymatic testing (TSD).

ACOG only
- Concurrent testing of patient and partner if patient of “greater” gestational age (>14 wks)
Differing Practice Guidelines: Ashkenazi Jewish Diseases (AJD)

Screening for other AJD

- ACMG: Should be offered for Fanconi anemia C, Niemann-Pick A, Bloom, mucolipidosis IV, Gaucher
- ACOG: Patients may inquire about other screening and information can be made available about testing for these disorders
- ACMG: Add’l disorders may be eventually added to recommendations if >90% DR and >1% allele frequency
Cystic Fibrosis

- Most common autosomal recessive disorder among Caucasians (1/3300)

- ~1/25 Caucasians *with no family history* is a carrier of CF

- 80% of children with CF are born to parents with no prior history of the disease
Testing for CF by genetic mutation analysis

- Nearly 2000 gene mutations identified
- Standard recommendation is a 23 mutation panel
  - Detects 94% Ashkenazi, 88% other Caucasian carriers
  - Detection rate and prevalence of disease both lower in other ethnic groups
<table>
<thead>
<tr>
<th>Group</th>
<th>Carrier risk</th>
<th>Detection rate</th>
<th>Carrier risk w/neg test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi</td>
<td>1/24</td>
<td>94%</td>
<td>1/380</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1/25</td>
<td>88%</td>
<td>1/200</td>
</tr>
<tr>
<td>Hispanics</td>
<td>1/58</td>
<td>72%</td>
<td>1/200</td>
</tr>
<tr>
<td>African-Am</td>
<td>1/61</td>
<td>64%</td>
<td>1/170</td>
</tr>
<tr>
<td>Asian-Am</td>
<td>1/94</td>
<td>49%</td>
<td>1/180</td>
</tr>
</tbody>
</table>

*Risks only apply with NEGATIVE family history!
CF genetic mutation analysis

- Original recommendation for 25 mutation panel
  - Present in at least 0.1% of cases of classical CF
  - Goal to screen for severe, classical phenotype
- With experience, 2 mutations removed as they caused mild or atypical disease
- Adding additional mutations is of limited benefit, as each new mutation typically rare
- Rare mutations are often of uncertain clinical significance
CF Screening Dilemma

- Drive toward expanded panels drive in part by purported better detection in racial/ethnic minorities
  - Original papers biased toward Caucasian and Ashkenazi groups who were better studied at the time
- Unclear if better detection is possible
  - Hispanic and African-Americans are genetically very diverse
CF mutation analysis

- Many of these additional mutations:
  - Are rare
  - Cause mild or atypical CF (sinusitis, nasal polyps)
  - Cause uncertain phenotype
  - Add little to detection rate
  - Increased detection almost entirely due to mutations that are inconsequential or of uncertain significance

- 100 mutations is NOT 4 times better than 23!

*Rohlf et al, Clin Chem 2011; Strom et al, Genet Med 2011*
CF mutation analysis

- CF testing is now a high volume service at commercial laboratories
- Many thousands of cases/week
  - Major source of revenue
- Highly competitive
  - Aggressive marketing based on # of mutations
CF Screening Dilemma

- Trade-off between public health/population based recommendations and individual patient preferences
- Some patients can understand nuances and limitations of rare and uncertain events
- But MOST struggle to understand even relatively straightforward genetic information
CF Screening Dilemma

Offering expanded panels requires:

- Understanding the significance of additional mutations detected by these panels
- Understanding the variability in reported phenotypes
- Being prepared to explain this to a patient when such is identified
Fragile X Syndrome

• Most common inherited form of mental retardation
  ▪ MR, joint laxity, tall stature, large jaw, characteristic faces, hyperactive behavior

• Most common single gene defect associated with autism

• 1/4000 males and 1/8000 females affected

• Carrier frequency 1/157

Berkenstadt et al, 2007
Fragile X Syndrome: Other features

Associated with a broad spectrum of clinical features:

- Late onset tremor/ataxia syndrome
- Premature ovarian failure
- Female infertility
- Psychiatric disease
- Autism
Fragile X Syndrome

Testing for Fragile X recommended for:

- Infertile women, esp with elevated FSH
- Egg and sperm donors
- Patients with a personal or family history of MR or developmental disabilities
- Patients with a personal or family history of autism

McConkie-Rosell et al, 2007
Fragile X Syndrome

- At present, population screening is not recommended
  - This is being debated
- Common form of MR, genetic test available, severe phenotype
- But the genetic counseling is complex
Carriers have a “premutation” that can expand to a “full mutation”

Full mutation in males and some females causes fragile X syndrome
- Outcome in females is unpredictable, from typical fragile X syndrome to a normal outcome
Fragile X

- Premutation only expands during female meiosis
- Premutation can cause some clinical symptoms
  - Infertility, premature ovarian failure in females
  - Late onset tremor / ataxia syndrome in males
Practice Guidelines: Fragile X

ACOG + ACMG basically in agreement about carrier screening indications:

- Family history of FraX, unexplained developmental delay, autism, premature ovarian insufficiency or increased FSH <40yo
- **ACOG** specifies women who request FraX screening should be offered testing after genetic counseling
- **ACMG** specifies testing should be offered with family history of cerebellar ataxia and intention tremor of unknown origin
- Population screening is not recommended by either
Carrier Screening for Spinal Muscular Atrophy (SMA)
Spinal Muscular Atrophy

- Severe hereditary neuromuscular disorder
- Degeneration of alpha motor neurons in spinal cord, resulting in proximal muscle weakness and paralysis
  - Several types of varying severity
  - Type I is most severe; usually results in death by age 2 from respiratory failure
Spinal Muscular Atrophy

- Autosomal recessive
- Second most common severe AR disorder after cystic fibrosis
- Most common inherited cause of early childhood death
- ~1/10,000 live births, 1/40-60 carrier frequency
Molecular Testing for SMA

• Homozygous deletion in exon 7 of SMN1 gene detects 95% of affected
• This mutation analysis cannot identify SMA carriers with heterozygous deletions
• Gene dosage analysis required for carrier testing
Complexities of Carrier Testing for SMA

- Negative screen reduces but does not eliminate risk (detects ~90%)

- Type 1 accounts for 70% of cases, type II and III for 30%; carrier testing does not predict type
Practice Guidelines: Spinal Muscular Atrophy

ACOG + ACMG have quite different opinions on SMA screening

ACMG:
• Carrier testing should be offered to all couples regardless of race or ethnicity

ACOG:
• Screening for SMA is not recommended for general population
• Screening should be offered to those with a family history of SMA, or if requested, after genetic counseling
Carrier testing is offered for disorders with a similar carrier frequency

SMA fits criteria for population-based screening (severe, high frequency, availability of test, availability of prenatal dx, access to GC)

Prior et al, Genet in Med, 2008
ACOG Committee on Genetics (2009)

- “Laboratories and advocacy organizations are promoting widespread screening”
- “…prenatal screening for SMA is not recommended in the general population at this time.”
- GC and screening should be offered to:
  - Persons with a family history
  - Those who request it
ACOG Committee on Genetics

Concerns include

- Relatively low incidence
- Complexities of molecular testing and interpretation
- Lack of genotype/phenotype correlation
- Difficulties in providing genetic counseling services
SMA Carrier Testing

Levi et al, 2012, AJOG/SMFM

- N=365 patients were offered both SMA and CF testing
- Far fewer had SMA screening performed:
  
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>SMA:</td>
<td>5.2%</td>
</tr>
<tr>
<td>CF:</td>
<td>80.3%</td>
</tr>
</tbody>
</table>
Universal Carrier Screening

- Universal carrier screening allows testing for many (>100) disorders at once
- This is relatively inexpensive ($99-299)
- Should it be offered to everyone?
About Counsyl

We have developed a single genomic test that replaces 100+ expensive assays

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloom Syndrome</td>
<td>$167</td>
</tr>
<tr>
<td>Canavan Disease</td>
<td>$473</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>$506</td>
</tr>
<tr>
<td>Familial Dysautonomia</td>
<td>$334</td>
</tr>
<tr>
<td>Fanconi Anemia</td>
<td>$167</td>
</tr>
<tr>
<td>Gaucher Disease</td>
<td>$467</td>
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<tr>
<td>Glycogen Storage Disease Type Ia</td>
<td>$283</td>
</tr>
<tr>
<td>Maple Syrup Urine Disease Type 1B</td>
<td>$557</td>
</tr>
<tr>
<td>Mucolipidosis IV</td>
<td>$279</td>
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<tr>
<td>Niemann-Pick Disease Type A</td>
<td>$337</td>
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<tr>
<td>Spinal Muscular Atrophy</td>
<td>$700</td>
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<tr>
<td>Tay-Sachs Disease</td>
<td>$473</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$4,743</strong></td>
</tr>
</tbody>
</table>

It has reduced the cost of carrier testing by literally one hundred fold
## Disease List

<table>
<thead>
<tr>
<th>Left Column</th>
<th>Right Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC8-related Hyperinsulinism</td>
<td>Hexosaminidase A Deficiency (Including Tay-Sachs Disease)</td>
</tr>
<tr>
<td>Achromatopsia</td>
<td>* HFE-associated Hereditary Hemochromatosis</td>
</tr>
<tr>
<td>Alkaptonuria</td>
<td>Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency</td>
</tr>
<tr>
<td>Alpha-1 Antitrypsin Deficiency</td>
<td>Hurler Syndrome</td>
</tr>
<tr>
<td>Alpha-Mannosidosis</td>
<td>Hypophosphatasia, Autosomal Recessive</td>
</tr>
<tr>
<td>Andermann Syndrome</td>
<td>Inclusion Body Myopathy 2</td>
</tr>
<tr>
<td>ARSACS</td>
<td>Isovaleric Acidemia</td>
</tr>
<tr>
<td>Aspartylglucosaminuria</td>
<td>Joubert Syndrome 2</td>
</tr>
<tr>
<td>Ataxia With Vitamin E Deficiency</td>
<td>Krabbe Disease</td>
</tr>
<tr>
<td>Ataxia-Telangiectasia</td>
<td>Limb-Girdle Muscular Dystrophy Type 2D</td>
</tr>
<tr>
<td>Autosomal Recessive Polycystic Kidney Disease</td>
<td>Limb-Girdle Muscular Dystrophy Type 2E</td>
</tr>
<tr>
<td>Bardet-Biedl Syndrome, BBS1-related</td>
<td>Lipoamide Dehydrogenase Deficiency</td>
</tr>
<tr>
<td>Bardet-Biedl Syndrome, BBS10-related</td>
<td>Long Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency</td>
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<tr>
<td>Biotindase Deficiency</td>
<td>Maple Syrup Urine Disease Type 1B</td>
</tr>
<tr>
<td>Bloom Syndrome</td>
<td>Medium Chain Acyl-CoA Dehydrogenase Deficiency</td>
</tr>
</tbody>
</table>
Carrier frequencies for recessive disorders (Lazarin et al., 2013)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency (1 in)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1-Antitrypin deficiency</td>
<td>13.1</td>
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<tr>
<td>Cystic Fibrosis</td>
<td>27.8</td>
</tr>
<tr>
<td>DFNB1 (non-syndromal deafness <em>GJB2</em>)</td>
<td>42.6</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>57.1</td>
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<tr>
<td>Familial Mediterranean fever</td>
<td>64.2</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>68.2</td>
</tr>
<tr>
<td>Sickle cell disease/beta-thalassemia</td>
<td>69.6</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>76.7</td>
</tr>
<tr>
<td>Factor XI deficiency</td>
<td>92.0</td>
</tr>
<tr>
<td>Achromatopsia</td>
<td>97.5</td>
</tr>
</tbody>
</table>
ECS Methods

• Can include genotyping or sequencing

• Genotyping:
  ▪ Includes only selected variants
  ▪ Significantly generally known

• Sequencing:
  ▪ Analyzes entire coding region
  ▪ Identifies more variants of uncertain significance
Universal Carrier Screening

Pro
- Cost effective
- Efficient
- Allows universal screening without regard to ethnicity

Con
- Too many unexpected findings (35% or so)
  - Need to screen the partner in all of these
- Disorders rare, esoteric, complex to explain
Universal Screening

• With advances in genetics, paradigm for testing will have to change from methodical, single disorder approach to broader screening

• Counseling by necessity will be more generic
  - “Do you want testing for birth defects?”
  - “Outcomes vary widely but generally none are desirable.”
  - “Not everything is detected by these tests.”
ACMG Position Statement on Universal Carrier Screening, 2013

- Most at-risk patients would consider prenatal diagnosis
- Specific consent must be obtained for adult onset disorders
- Genes, mutations, and mutation frequencies must be known so risks can be calculated
- Correlation between mutation and phenotype must be known
- Lab quality standards must be followed
American College of Medical Genetics and Genomics:

- “Most at-risk patients … would consider having a prenatal diagnosis.” [MTHFR]
- “Disorders characterized by variable expressivity or incomplete penetrance … or associated with a mild phenotype should be optional…” [hemochromatosis]
American College of Medical Genetics and Genomics:

• “Validated clinical association between mutation(s) detected and severity of the disorder” [cystic fibrosis]
Current Commentary

Expanded Carrier Screening in Reproductive Medicine—Points to Consider

A Joint Statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine

Obstet Gynecol 2015
Timing of ECS

- Ideally performed before pregnancy
  - Sequentially or concurrently
- Timing in pregnancy depends on
  - Gestational age, partner availability, patient preferences
- Genetic counseling recommended for patients with significant family history
  - Familial mutation may not be on panels
ECS: Consent

- Carrier screening is voluntary
- Conditions vary in severity
- Risk assessment depends on accurate paternity
- A negative screen does not eliminate risk
- Expanded screening includes many conditions and it is common to carry $\geq 1$
- Some individuals carry two mutations for a disorder but are asymptomatic
Limitations of ECS

- Contains most conditions recommended in current guidelines, BUT
- Molecular methods may not be as accurate as biochemical tests for some conditions
  - Hemoglobinopathies (MCV, electrophoresis)
  - Tay Sachs (hex A enzyme levels)
Recommendations

• Have a clear policy for screening for your practice

• Have a rationale for your approach

• Have a clear follow up or referral plan for patients with positive results
Selecting Genetic Testing

- Whole exome sequencing
- Chromosomal microarray
- Gene panels
- Single gene tests

- Broader coverage

- More specific testing, greater certainty of diagnosis
Genetics vs. genomics

Genomic tests – examining the entirety of the genetic material

Very low resolution: karyotype

Low resolution: microarray

High resolution: Whole genome sequencing

Single gene test (ie CF)
Cell free DNA: lowest resolution

<table>
<thead>
<tr>
<th>CHROMOSOME</th>
<th>RESULT</th>
<th>PROBABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21 (T21)</td>
<td>Low Risk</td>
<td>Less than 1/10,000 (0.01%)</td>
</tr>
<tr>
<td>Trisomy 18 (T18)</td>
<td>Low Risk</td>
<td>Less than 1/10,000 (0.01%)</td>
</tr>
<tr>
<td>Trisomy 13 (T13)</td>
<td>Low Risk</td>
<td>Less than 1/10,000 (0.01%)</td>
</tr>
<tr>
<td>Fetal Sex</td>
<td>Male Fetus</td>
<td>Greater than 99/100 (99%)</td>
</tr>
<tr>
<td>X,Y Analysis</td>
<td>XY</td>
<td>Greater than 99/100 (99%)</td>
</tr>
</tbody>
</table>
Recommendations

• Provide written information
• Explain what is recommended by professional organizations and what is not
• Emphasize that not everything is detected
Recommendations

• Refer to genetic counseling those who want detailed information
  ▪ Patients who request additional screening beyond what you are comfortable with

• Low threshold to provide additional testing on patient request
Final Thoughts

“…..the foremost purpose of prenatal screening is not to reduce the incidence of genetic disease but to fulfill a couple’s reproductive goals.”

Thank You!