Overview of CF, CFTR genotyping, and some MCADD

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Wadsworth Center
Welcome to the Clinical and Functional Translation of CFTR (CFTR2) website

CFTR2 is a website designed to provide information about specific cystic fibrosis (CF) mutations to patients, researchers, and the general public. For each mutation included in the database, the website will provide information about:

- Whether the mutation causes cystic fibrosis when combined with another CF-causing mutation, and
- Information about the sweat chloride, lung function, pancreatic status, and pseudomonas infection rates in patients in the CFTR2 database with this mutation.

How to use this website

For patients and family members
This website provides information about specific CF mutations only. This website is intended for members of the general public who want to find out what we currently know about specific mutations related to cystic fibrosis. This includes:
- Cystic fibrosis (CF) patients,
- Family members of CF patients,
- People who are carriers of a CF mutation, and
- Parents whose baby has just been diagnosed with CF through newborn screening.

For health care providers/scientists
This section provides scientific and medical descriptions intended for CF researchers, health professionals, and members of the general public that are looking for more in-depth, research-related information. Patients and their families are encouraged to visit the section “For patients and family members” first.

WHAT THIS SITE IS NOT INTENDED TO DO:
- This website is not intended to help diagnose anyone with CF.

For more information about CF, click here.

Note: If you have questions about any of the information contained in this website, please consult your doctor.

Enter the site for CF patients, family members, or carriers
Enter the site for health care providers/scientists
Cystic Fibrosis

- Genetic condition – 1/3,500 births; 35,000 individuals in US
- Progressive lung disease; babies have other issues

Median Predicted Survival: 37 years
Median Age at Death: 26 years

Patient Registry, Cystic Fibrosis Foundation, 2008, Bethesda MD, USA (N=c.25,000)
Multi Organ Dysfunction in CF

- **Sinuses** – Sinusitis, nasal polyps
- **Lung** – Endobronchitis, bronchiectasis
- **Pancreas** – Exocrine Insufficiency, CF Related Diabetes
- **Intestine** – Meconium ileus; constipation
- **Liver** – Focal sclerosis
- **Vas Deferens** – failure to develop **CBAVD**
- **Sweat gland** – salt-losing dehydration

Adapted from Welsh and Smith, Sci Am, 1995
Evolution of Cystic Fibrosis Screening

- Pan-ethnic
- Frequency 1:3,300 to 1:90,000 worldwide
- Gene described in 1989
- Prenatal screening very early 90's; linkage
- 1997 NIH Consensus Conference; follow-up
- ACMG carrier screening panel 2001; 2004
- Google search CF screening: 14,700,000 hits (7/2/13)

Balancing Carrier Screening Principles v. Newborn Screening
Introduction of Mutation Analysis to CF NBS

- CF Mutation identified in 1989
Carrier screening should be standard of care to pre-pregnant Caucasian women.

Carrier screening should be offered to all other pre-pregnant women.

“On the basis of a preponderance of evidence, the health benefits to children with CF outweigh the risk of harm and justify screening for CF.”

“Newborn screening systems should ensure parental and provider education…”
Status of CF NBS in 2004

- **Universally required**
- **Universally offered, but not required**

*Slide courtesy P. Farrell*
Current Status of CF NBS (2006)

- Universally required
- Universally offered, but not required
- Offered to select populations or by request
- Advanced planning stages
- Considering various options
- Required but not yet implemented
- No information on current intentions
Current Status of CF NBS (2009)

- Universally required
- Approved and scheduled

12/09?
By 2010, Newborn Screening was the Most Common Diagnostic Indication

U.S. CF Foundation Registry

All new diagnoses reported to CFF in each year

Presented at NACFC, November 2011, Anaheim
Age of Diagnosis Has Decreased with Newborn Screening

U.S. CF Foundation Registry

All new diagnoses reported to CFF in each year

By end of 2010
83% of CF dx’d
by NBS or MI

Presented at NACFC, November 2011, Anaheim
NBS infants are Less Likely to be Malnourished
(weight for age < 3rd percentile)

Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41)
Infants NBS-screened for CF have Fewer Hospitalizations

Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41)
IRT / IRT

- Newborns receive 2 newborn screen tests
  - 1\textsuperscript{st} before hospital discharge
  - 2\textsuperscript{nd} at 2 week well baby check (mandated or extra sample collected)

- IRT is tested on both newborn screen blood spots

- If both IRTs are elevated, child is recalled for a sweat test (e.g. cutoffs at 100ng/ml and 70ng/ml)

- No genetic testing is performed – no carriers are identified
Enzyme-Linked ImmunoSorbent Assay (ELISA)
IRT / DNA

- Newborns receive 1 newborn screen test
- IRT is tested on dried blood spot
- If IRT is elevated, same sample is tested for a panel of CFTR mutations.
- If 1 or more CFTR mutations are identified, child is recalled for a sweat test
  - 2 mutations – presumptive positive (sweat test)
  - 1 mutation – possible CF (sweat test) **reveals carrier status

Variations on this general theme: an also have IRT / IRT / DNA; IRT / DNA / EGA
Goals for NBS Tests in CF

- Minimize false negatives (Sensitivity); rules-in dx
  - 60% of cases have 2 mutations
- Balance the number of false positives (PPV)
- Provide a more specific diagnosis, i.e. DNA
- *Minimize the need for genetic counseling for detection of carriers*
- Reduce parental stress
  - Reduce the time to a diagnosis
  - Reduce the number of children/parents recalled for testing
  - Provides familial genetic information
- Reduce costs of screening and follow-up
Selection of CFTR Mutations

• Only mutations known to cause CF should be included in a panel

• 23-mutation ACMG
  – High degree of sensitivity
  – All mutations known to cause disease (special case R117H*)

• Population considerations

****Limited by vendor panels****

CLSI. Newborn Screening for Cystic Fibrosis; Approved Guideline. CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011
Allele Frequencies of CFTR Mutations From the ACMG-23 Panel Reported in Cohorts Detected Through CF NBS

<table>
<thead>
<tr>
<th>Allele</th>
<th>CA* (23)</th>
<th>MA* (24)</th>
<th>NY* (24)</th>
<th>CO* (25)</th>
<th>WI* (26)</th>
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<tr>
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<td>67.9</td>
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<td>3.2</td>
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<tr>
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<td>3.1</td>
<td>1.4</td>
<td>1.4</td>
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<tr>
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<td>0.4</td>
<td>0.5</td>
<td>1.6</td>
<td></td>
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<tr>
<td>R553X</td>
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<td>0.4</td>
<td>0.9</td>
<td>1.8</td>
<td>2.4</td>
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<tr>
<td>3120+1G&gt;A</td>
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<td>0.5</td>
<td></td>
<td>2.4</td>
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<td>2.4</td>
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<tr>
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<td>0.9</td>
<td>0.5</td>
<td></td>
<td>2.4</td>
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<tr>
<td>R334W</td>
<td>2.5</td>
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<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R117H</td>
<td>†</td>
<td>4.0</td>
<td>0.9</td>
<td></td>
<td>‡</td>
</tr>
<tr>
<td>R347P</td>
<td></td>
<td></td>
<td>0.5</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

* CA = California; MA = Massachusetts; NY = New York; CO = Colorado; WI = Wisconsin.
† Detection of this allele trans to a disease-causing mutation was excluded from percentages reported by these authors, but would have been > 1%.
‡ Not tested in this mutation panel.

CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guideline* CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011.
Fig. 1. Diagnóstico molecular de la mutación ΔF508 en afectados con Fibrosis Quística. M: Marcador de peso molecular (2174 dígerido con Hae III). Cs: Control de sistema (mezcla de reacción sin ADN). C+1: Control positivo 1 (afectado con Fibrosis Quística, homocigoto ΔF508/ΔF508). C+2: Control positivo 2 (afectado con Fibrosis Quística, Heterocigoto compuesto ΔF508/X). C−: Control negativo (individuo sin Fibrosis Quística). A1: Patrón molecular de afectado con Fibrosis Quística homocigoto ΔF508/ΔF508. A2: Patrón molecular de afectado con Fibrosis Quística heterocigoto compuesto ΔF508/X. A3: Patrón molecular de afectado con Fibrosis Quística sin la mutación ΔF508 (X/X). Heteroduplex: Dos bandas de mayor tamaño, las cuales son el resultado del apareamiento del fragmento de 95 y de 98 pb.
Restriction Fragment Length Polymorphism

Example not CF but this was used for common small panels early on
ARMS PCR analysis

2-tube; 2 primers each

1-tube; 4 primers


ABI 3100

Gene fragment analysis
Sequence analysis

Cystic Fibrosis Profile

Software calls alleles
2 abnormal alleles
RED !!!
ABI ASSAY

32 Mutations
Single Tube
PCR: OLA
15 Primer pairs
66 site probes
The Invader™ assay from Third Wave Technologies, Inc. relies upon the specificity of the Cleavase® enzymes, which recognize only the invasive complex, permitting discrimination of single base changes.
42 Variants
ACMG/ACOG 23
V520F
3876delA
394delTT
R347H
I148T; reflex 3199del6
1078delT
3905insT
S549N
Y122X
Y1092X
Y1092X(C>G)
S549R(T>G)
2183AA>G
S549R(A>C)
D1152H
3849 + 4A>G
E60X
Q493X
D1270N
Nucleic Acid Extraction and Purification
A optimal input quantity of 50ng (range of 10 ng to 1.5 ug) per sample is required to perform the assay.

Step 1 - Multiplex PCR Reaction will make multiple copies of multiple DNA targets within the CFTR gene.

Step 2 - Amplicon Treatment
Enzymatic treatment of amplified PCR products cleaves unused reagents (primers and dNTPs) left over after PCR.

Step 3 - Allele-specific primer extension (for CF)
The amplified DNA is mixed with short sequences (TAG primers) of DNA specific to each target. If the target is present, the primer will bind and will be lengthened through a process called Allele specific extension. During this extension, a reporter label is incorporated.

Step 4 - Bead Hybridization
Color-coded beads are added to identify the tagged primers. Attached to each differently colored bead is an anti-TAG sequence specific to one of the extended TAG primers. Each anti-TAG only binds to the complementary TAG sequence on the primer.

Step 5 - Addition of Reporter Molecule
The reporter solution is the Streptavidin, R-Phycoerythrin conjugate and will be used to detect the target.

Step 6 - Data Acquisition on Luminex Analyser
Samples are then placed in a Luminex xMAP® instrument where beads are read and analyzed by lasers. The lasers identify the color of the bead and the presence or absence of the labeled target. For each sample, these signals are interpreted by the xTAG Data Analysis Software to determine whether the wild-type and/or mutant alleles for each of the variations have been detected.
xTAG® Cystic Fibrosis (CFTR) 39 kit v2*

<table>
<thead>
<tr>
<th>Mutation</th>
<th>ACMG recommend mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508</td>
<td>A455E</td>
</tr>
<tr>
<td>ΔI507</td>
<td>1717-1G&gt;A</td>
</tr>
<tr>
<td>G542X</td>
<td>R560T</td>
</tr>
<tr>
<td>G85E</td>
<td>R553X</td>
</tr>
<tr>
<td>R117H</td>
<td>G551D</td>
</tr>
<tr>
<td>621+1G&gt;T</td>
<td>1898+1G&gt;A</td>
</tr>
<tr>
<td>711+1G&gt;T</td>
<td>2184delA</td>
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<tr>
<td>R334W</td>
<td>2789+5G&gt;A</td>
</tr>
<tr>
<td>R347P</td>
<td>3120+1G&gt;A</td>
</tr>
</tbody>
</table>

16 most common additional mutations recommended mutations covered

List of mutations or variants identified in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.
*For In Vitro Diagnostics Use Only,

xTAG® Cystic Fibrosis (CFTR) 60 Kit v2*

<table>
<thead>
<tr>
<th>Mutation</th>
<th>ACMG recommend mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR del2,3</td>
<td>2055del9&gt;A</td>
</tr>
<tr>
<td>W1089X</td>
<td>S1196X</td>
</tr>
<tr>
<td>1677delTA</td>
<td>935delA</td>
</tr>
<tr>
<td>D1152H</td>
<td>2143delT</td>
</tr>
<tr>
<td>R1158X</td>
<td>K710X</td>
</tr>
<tr>
<td>G178R</td>
<td>G330X</td>
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<tr>
<td>3791delC</td>
<td>Q890X</td>
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<tr>
<td>L206W</td>
<td>L1066C</td>
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<tr>
<td>E60X</td>
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<tr>
<td>R75X</td>
<td>406-1G&gt;A</td>
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<tr>
<td>Q493X</td>
<td>Q493X</td>
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</table>

Broad Ethnic Coverage

CF MUTATION DETECTION RATE (%)*

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Incidence of CF*</th>
<th>Carrier Frequency*</th>
<th>ACMG RECOMMENDED MUTATIONS ONLY</th>
<th>xTAG® Cystic Fibrosis 39 Kit v2</th>
<th>xTAG® Cystic Fibrosis 60 Kit v2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>1 in 3,200</td>
<td>1 in 25</td>
<td>88.3</td>
<td>89.7</td>
<td>90.6</td>
</tr>
<tr>
<td>Hispanic Americans</td>
<td>1 in 9,500</td>
<td>1 in 46</td>
<td>71.7</td>
<td>73.4</td>
<td>83.7</td>
</tr>
<tr>
<td>African Americans</td>
<td>1 in 15,300</td>
<td>1 in 65</td>
<td>64.5</td>
<td>68.6</td>
<td>72.5</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>1 in 3,300</td>
<td>1 in 25</td>
<td>94.0</td>
<td>94.0</td>
<td>95.9</td>
</tr>
<tr>
<td>Asian American</td>
<td>1 in 32,100</td>
<td>1 in 90</td>
<td>48.9</td>
<td>54.5</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Multiplex Ligation Probe Amplification Assay

- Exon 2-3 deletion
- "50Kb deletion"
- Can be difficult to optimize
- Paucity of controls
- 1-5% of mutations
“however, with people like Francis Crick around it was difficult to ignore nucleic acids or to fail to realize the importance of sequencing them.”
Mix deoxynucleotides with ddA, ddT, ddC*, ddG
4 lanes per person/fragment
~200 readable bases

Chop up the human genome
Make a library of fragments
Sequence billions of bases
Multiplexing multiple people
Millions of ‘reads’

Mix deoxynucleotides with ddA, ddT, ddC*, ddG
1 scan per person/fragment
~800 readable bases
Fatty Acid Oxidation Disorders (FAOs)

- Genetically determined inborn errors of metabolism
- Myopathy, cardiomyopathy, and SIDS-like presentation
- Initial presentation: hypoketotic hypoglycemia
- Abnormal response to fasting and/or infectious disease stress
- Fasting >12 hours exhaust glycogen stores and mobilize fatty acids for energy
- Dehydrogenases have overlapping specificities for chain length
## Acyl-CoA Dehydrogenases / Substrate Specificities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate Chain length</th>
<th>Deficiency Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>short chain (SCAD)</td>
<td>C4-6</td>
<td>Rare</td>
</tr>
<tr>
<td>medium-chain (MCAD)</td>
<td>C6-12</td>
<td>Common (1:10,000)</td>
</tr>
<tr>
<td>very long-chain (VLCAD)</td>
<td>C12-16</td>
<td>Rare</td>
</tr>
</tbody>
</table>

- MCAD is one of three mitochondrial Acyl-CoA dehydrogenases
- The homotetramer enzyme catalyzes the initial step of the mitochondrial fatty acid beta-oxidation pathway
- MCAD substrates are fatty acyl CoAs with acid chain length of C6-C12
- MS/MS is used to establish an acylcarnitine profile
- Elevation of octanoylcarnitine (C8) is the main marker
- C6 and ratio C8:C2 help as discriminators or secondary markers
Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)

- 1/6,000-10,000 Caucasian births
- Most common/Classic FAO disorder
- Present as hypoketotic/hypoglycemic (Reye Sx) and/or myo/cardio-myopathy, hypotonia, CHF, arrhythmia, SIDS
- Episodic illness 6m–2y after 12 h fasting or intercurrent infectious disease (vomiting / lethargy / seizures / coma)
- Most patients normal between episodes / some hypotonic or poor muscle strength
NYSDOH-NBSP MCADD MS/MS Detection

MCAD Normal Specimen
Genetics

- Autosomal recessive
- Gene (ACADM) on chromosome 1p31.1
- 12 exons, 421 amino acids
- c.985A>G (p.Lys329Glu = p.K304E) most common mutation
- C8>0.8 μmole/L are referred for DNA analysis
- NYS-NBSP uses FRET/RT-PCR to detect p.K329E
- Complete gene sequencing
Lightcycler by Roche

- uses capillaries for PCR
- cycling in 25 minutes,
- data analysis in 40 minutes

Roche

Wadsworth Center
Roche

Wadsworth Center
FRET analysis for the most common ACADM Mutation

c.985A>G = p.K329E (K304E)
<table>
<thead>
<tr>
<th>c.DNA</th>
<th>Protein</th>
<th>Homo</th>
<th>Het</th>
<th>Carrier Freq</th>
<th>Allele Freq</th>
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<td>19</td>
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Acknowledgements

- CDC and APHL for inviting me to talk today
- Marci Sontag, Ph.D., University of Colorado at Denver
- Carlos Saavedra, M.D., Wadsworth Center, DOH