



# Updates in Molecular Diagnostics and Carrier Screening for Cystic Fibrosis

**New Extended Client Service Hours**  
**8:00 am – 8:00 pm EST**

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Ambry Webinar Series

# AGENDA

- **History and Background of CF Molecular Diagnostics**
- **Carrier screening panels**
- **Explanation and significance of R117H/Poly T/TG repeat**
- **Statistical Information**
- **Newborn screening overview and follow up recommendations.**

# HISTORY

- 1989 CFTR gene cloned
  - deltaF508 = ~70% of CFTR mutations
    - 6 mutation panel
    - 12 mutation panel etc.

# ACOG/ACMG/NHGRI - 2001

**Laboratory Standards and Guidelines for Population-based Cystic Fibrosis Carrier Screening** *Genetics in Medicine*, 2001, 3, #2: 149-154

- Offered to people with a family history of CF and to reproductive partners of persons with CF,
- Offered to couples where one or both partners are Caucasian and are planning a pregnancy or seeking prenatal care, and.
- Available with appropriate information about limitations to couples in other racial or ethnic groups who are at lower risk and for whom testing is less effective (e.g., Hispanics, African Americans, Asian Americans).

# ACOG/ACMG 25 Mutation Panel

DeltaF508	DeltaI507	G542X	G551D
W1282X	N1303K	R553X	621+1 G>T
R117H	1717-1 G>A	A455E	R560T
R1162X	G85E	R334W	R347P
711+1 G>T	1898+1 G>A	2184delA	1078delT
3120+1 G>A	2879+5 G>A	3659delC	I148T
3849+10kb C>T			

# CFTR Mutation Frequency

- >1500 known mutations
- DeltaF508 = ~30-70% depending on ethnicity
- 99% of all mutations occur < ½ of 1%

# Our History

- **Clinical diagnostic laboratory since 2000**
  - **Offering full gene analysis for *CFTR* for over 10 years**
  - **Over 30,000 patient samples analyzed for the *CFTR* gene**
  - **Over 11,000 deletion duplication studies for *CFTR***
  - **Recognized as the leading experts for mutation interpretation**

# Why is full sequence analysis important?

- More than 1,500 mutations have been described for the *CFTR* gene
- Uncommon mutations can cause severe disease
- Only 6 mutations other than deltaF508 occur at frequencies over 1% in the CF population
- Carrier screening mutation panels were not designed for diagnostics

# When is full sequence analysis indicated?

- **Diagnostic testing**
- **High-risk carrier screening**
- **Men with CBAVD**
- **Follow-up for positive newborn screening**
- **Chronic or hereditary pancreatitis**

# Summary

- **The mutation spectrum is diverse in all ethnic groups studied to date**
- **The frequencies of most mutations are low**
- **Uncommon mutations are present in patients with mild and severe phenotypes**
- **Carrier screening panels are of limited use in CF diagnostics**
  - **ACMG-23 panel screening would have only detected 37% (African A.), 22% (Asian), and 2.4% (Asian Indian) of the variants identified by Full Gene Analysis!**

# Our Experience: *CFTR* Deletions & Duplications

- **11,065** patients underwent analysis
- **149 (1.35%)** patients carried a deletion or duplication
  - 141 patients with 1 deletion
    - 8 patients were homozygous deletions
- **24** Different Deletions
- **8** Different Duplications

# The Ambry Experience:

## CFTR Gross Deletions & Duplications

- **2181** patients referred for diagnostic testing who had one mutation on sequencing and underwent Del/Dup studies
- **98/2181 (4.5%)** patients carried a deletion or duplication as their second deleterious mutation
- The greater the clinical suspicion of CF the greater the likelihood the second mutation is a deletion

# R117H and I148T:

How do I counsel regarding these findings?

- I148T
  - Real Mutation or Not?
- R117H
  - Mild mutation even without a 5T
  - Poly T and TG repeat information is critical

# I148T

- Not a mutation, just a polymorphism
- 123 patients identified to carry the I148T
  - 6 (4.8%) with 3199del6
  - 4 (3.3%) with T1299I
- The Real Mutations are **3199del6** and **T1299I**

# R117H

This is a Mutation!

- **R117H**
  - It is a very mild mutation in the absence of the 5T
  - Poly T and TG repeat information is critical
  - In cis with 5T can be moderate to severe mutation
    - 5T with more TG repeats increases the severity of the mutation

# What Is a TG and Why Does It Repeat?

**CFTR TG Tract in intron 8 is adjacent to the poly T tract**

**Those individuals with 5T adjacent to either 12 or 13 TG repeats were substantially more likely to exhibit an abnormal phenotype than those with 5T adjacent to 11 TG repeats\*.**

\*Variation in a Repeat Sequence Determines Whether a Common Variant of the Cystic Fibrosis Transmembrane Conductance Regulator Gene Is Pathogenic or Benign, **Groman et. al., *Am. J. Hum. Genet.* 74:176–179, 2004.**

# Evolution of the CFTR sequencing test

- **When do you need to repeat testing?**
  - **If testing performed prior to 2006**
    - **Call for clarification**
- **Need to follow up**
  - **Testing parents to determine cis or trans**
- **Costs, How to order**

# Newborn Screening

- Other State Newborn Screening Programs
  - IRT/IRT
  - IRT/DeltaF508
  - IRT/Mutation Panel
- California Newborn Screening Program
  - IRT
  - 38 Mutation panel
  - Full sequence analysis

# Newborn Screening cont.

- **Follow Up**
  - **Know what is done in your state**
- **Is this CF?**
  - **CRMS v. CF v. CF carrier**
- **When to Sequence?**

# CF102 – The Ambry Carrier Screening Panel

CF102 detection rate compared to the rates of other CF tests

<u>Screening Panel</u>	<u>Detection Rate</u>
<b>Ambry CF Screening Panel (102 mutations)</b>	<b>91%</b>
ACOG/ACMG (23 mutations)	75.7%
Company B (97 mutations)	82.6%
Company C (100+ mutations)	83.7%

# The Development of the CF102 Panel

- **Selection of patient population to determine detection rate**

- To provide an unbiased patient population

- **Patient characteristics:**

- A patient population of 592 previously untested, clinically diagnosed Cystic Fibrosis patients for a total of 1,184 CFTR disease causing mutations.

- **Determination of detection rate:**

- This pool of 1,184 known disease causing mutations was used to determine the detection rate of all mutation panels.
- This cohort of patients is representative of a population of **3.6 million people** (larger than the population of 22 different states) (592x300x2)

# Known Mutation Linkage

- **DeltaF508** and **9T**
- **R668C** and **G576A** and **D443Y**
- **D1270N** and **R74W** and **V201M**
- **DeltaF508** and **I1027T**
- **R74Q** and **R297Q**
- **3849+10kbC>T** and **R668C**

# Mutations or Polymorphisms

•M470V (20,040)

•R75Q (1,426)

•R117H (544)

•T854T (12,723)

•Q1463Q (7,853)

•N1303K (180)



# Other Tests Available from Ambry

- Surfactant testing (*SFTB, SFTC, ABCA3*)
- Congenital Central Hypoventilation Syndrome (*PHOX2B*)
- Primary Ciliary Dyskinesia (*DNAI1, DNAH5*)
- Alpha-1-Antitrypsin (*SERPING1*)
- Idiopathic Pulmonary Fibrosis (*TERT, TERC*)
- Pulmonary Arterial Hypertension (*BMPR2*)

# QUESTIONS?

## Direct Contact Information

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