SEQUENCING RESULTS
INTERPRETATION AND REPORTING

RACHEL LEE, PH.D.

Gene Sequencing in Public Health Newborn Screening Meeting

February 17, 2017
NOW WHAT?

- Interpretation
  - Classification of variants
  - Database and other resources

- Reporting
  - Regulatory requirements
  - Clinical significance and recommendation
  - Timing of reporting
早安
Zao An
Early Safe
Buenos dias
Good Morning
No Molecular Genetics specialty – general requirements
Nonwaived
High complexity testing
LDTs
OTHER REGULATIONS AND GUIDELINES

- College of American Pathologists
- State specific, e.g. NY Clinical Laboratory Evaluation Program
- Professional Standards:
  - Clinical Laboratory Standards Institute
  - American College of Medical Genetics and Genomics
  - Association of Molecular Pathologists
TERMINOLOGY

- Mutation
- Polymorphism
- SNP, Single nucleotide polymorphism
Variant
ACMG STANDARDS AND GUIDELINES

- Workgroup consisted of ACMG, AMP, and CAP experts
- ACMG determined 5 categories to classify variants using standard terminology:
  - Pathogenic
  - Likely to be pathogenic
  - Uncertain significance
  - Likely to be benign
  - Benign

Richards, S. et al. 2015. Genetics in Medicine 17(5):405-424
RESOURCES

- Peer reviewed publication
- Database
  - General
  - Disorder specific
- Predictive program
- ACMG proposed criteria
PUBLICATIONS

- PubMed (Medline and Medline Plus)
- ScienceDirect
- SpringerLink
- Ovid, Ebscohost, ProQuest.
- Cochrane Library
- Google Scholar
- ........


PUBMED SEARCH EXAMPLE

Clinical expression of patients with the D1152H CFTR mutation.
PMID: 255683415
Similar articles

Genetic and electrophysiological characteristics of recurrent acute pancreatitis.
PMID: 25383785
Similar articles

Mechanisms of CFTR functional variants that impair regulated bicarbonate permeation and increase risk for pancreatitis but not for cystic fibrosis.
PMID: 25003378
Free PMC Article
Similar articles
GENERAL DATABASE

- ClinGen
- ClinVar
- OMIM
- Human Gene Mutation Database (HGMD)
- Exome Aggregation Consortium (ExAC)
- 1000 Genome Project
- Exome Variant Server
- dbSNP

............
DISORDER SPECIFIC DATABASE

- CFTR2, https://www.cftr2.org/
- HbVar, http://globin.bx.psu.edu/hbvar/menu.html
- ALD Mutation Database, http://www.x-ald.nl/
CLINVAR SEARCH EXAMPLE – ΔF508
Results for F508del

Variant F508del can be refereed to as F508del, Phe508del, 1653delCTT, or c.1521_1523delCTT

- The drug combination of Ivacaftor and lumacaftor (Orkambi) has been approved in some countries for certain individuals with this variant. Please contact your physician to discuss whether the combination of ivacaftor and lumacaftor is appropriate for you.

Summary Information

- **This variant causes CF** when combined with another CF-causing variant. (The other CF-causing variant does not have to be variant F508del. It can be a different variant that also causes CF.)
- **This variant causes pancreatic insufficiency** when combined with another variant that causes pancreatic insufficiency.
  - Patients with this variant will probably need to take oral pancreatic enzyme supplements every day.
  - The oral supplements help the patients' bodies to absorb the nutrients and vitamins contained in the food they eat.
  - The oral pancreatic supplements will not prevent patients from developing CF.
- There are 64,864 patients with this variant in the CFTR2 database.

For help interpreting this information, we recommend you watch this video overview: What is Cystic Fibrosis?
CLINVAR SEARCH EXAMPLE – D1152H

NM_000492.3(CFTR):c.3454G>C (p.Asp1152His)

Variation ID: 35867
Review status: criteria provided, multiple submitters, no conflicts

Interpretation
Clinical significance: Pathogenic
Last evaluated: Jul 18, 2016
Number of submission(s): 8
Condition(s):
- Bronchiectasis with or without elevated sweat chloride 1 [MedGen - OMIM]
- Cystic fibrosis [MedGen - Orphanet - OMIM]
- Hereditary pancreatitis [MedGen - Orphanet - OMIM]
- Congenital bilateral absence of the vas deferens [MedGen - Orphanet - OMIM]

Variant frequency in dbGaP
NM_000492.3(CFTR):c.3454G>C (p.Asp1152His)
GRCh37 Chr7:117264763

Sample count
Called variants: 3 of 8042
Potential variants: 33 of 40679

Called variants are samples submitted to dbGaP that have the variant allele. Potential variants are SRA runs that display the allele in at least 30% of the reads covering the position, and have 10 or more passing
Results for D1152H

Variant D1152H can be referred to as D1152H, p.Asp1152His, or c.3454G>C

- This variant has **varying consequences**.
- Some patients with this variant, combined with another CF-causing variant, have CF.
- Other patients with this variant, combined with another CF-causing variant, do not have CF.
- Because of this variability, it is **very important** that **clinical criteria alone** be used to determine whether a person with this variant has CF.
- Because the clinical manifestations of CF can vary over the course of a person’s lifetime, people who have this variant plus a variant that is known to cause CF should have periodic check-ups with their doctor even if they have no clinical signs or symptoms of CF at the present time.
- Clinical information shown below is taken only from patients in the CFTR2 database who have been diagnosed with CF.
  - There are other people with this variant who do NOT have CF. Information from these people is NOT included in the clinical information below, because these individuals are not followed at a CF center and are not part of the CFTR2 database. Therefore, the data below is not representative of every person with this variant.
  - **Patients with CF who have this variant are likely to be pancreatic sufficient.** This means they may not need to take oral pancreatic enzyme supplements every day.
  - There are 555 patients with this variant in the CFTR2 database.

For help interpreting this information, we recommend you watch this video overview *What is Cystic Fibrosis?*
CLINVAR SEARCH EXAMPLE – R31C

NM_000492.3(CFTR):c.91C>T (p.Arg31Cys)

Variation ID: 35893
Review status: criteria provided, conflicting interpretations

Interpretation:
Clinical significance: Conflicting interpretations of pathogenicity
Benign(1); Likely benign(1); Uncertain significance(3)
Last evaluated: March 3, 2016
Number of submission(s): 5
Condition(s): Cystic fibrosis

Variant frequency in dbGaP:
NM_000492.3(CFTR):c.91C>T (p.Arg31Cys)
GRCh37 Chr7:117144344
Sample count:
<table>
<thead>
<tr>
<th>Called variants</th>
<th>Potential variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 of 8679</td>
<td>129 of 40822</td>
</tr>
</tbody>
</table>
Results for **R31C**

Variant R31C can be referred to as R31C, p.Arg31Cys, or c.91C>T

- This variant **DOES NOT cause CF** when combined with another CF-causing variant.
- There may be patients in the CFTR2 database with this variant who have CF, but this variant is NOT THE CAUSE of their CF.
- There are 23 patients with this variant in the CFTR2 database.
- Based on:
  - clinical information obtained from these patients,
  - laboratory experiments performed on this variant, and
  - analysis of groups of healthy individuals that carry this variant,
  we conclude that this variant does not cause CF when combined with another CF-causing variant.

Most individuals with this variant (combined with another CF-causing variant) will be healthy. A small number of individuals may develop mild symptoms or be diagnosed with a CFTR-related disorder (CFTR-RD; see FAQs), but symptoms are not expected to be severe enough to meet the definition of CF. Some individuals with a CFTR-RD may be represented in the CFTR2 database because they are followed by CF clinics. In this case, R31C MAY be related to their symptoms. However, it is unlikely that these patients meet the criteria for a CF diagnosis.

For help interpreting this information, we recommend you watch this video overview What is Cystic Fibrosis?
Your Own Database

- CAP requirement

MOL.49575 Variant Database

The laboratory's database for the clinical significance of variants is recorded and updated as needed, when applicable.

- Keep an up-to-date database

- Track genotype-phenotype correlation and frequency of variants in your population
MAINTAINING YOUR OWN DATABASE

Data fields
- Gene name
- Nomenclature
- Classification
- Interpretation
- References
- Date of last update

Frequency of updates – data curation
PREDICTIVE PROGRAM

- SIFT
- PolyPhen-2
- Mutation Taster
- Condel
- PROVEAN
- GeneSplicer
- ..........
**Mutation Taster Example – D1152H**

**Prediction**

- **disease causing**

**Summary**

- amino acid sequence changed
- known disease mutation: rs75541969 (pathogenic)
- protein features (might be) affected

<table>
<thead>
<tr>
<th>analysed issue</th>
<th>analysis result</th>
</tr>
</thead>
<tbody>
<tr>
<td>name of alteration</td>
<td>D1152H</td>
</tr>
<tr>
<td>alteration (phys. location)</td>
<td>chr7:117254753G&gt;C <a href="#">show variant in all transcripts</a></td>
</tr>
<tr>
<td>HGNC symbol</td>
<td>CFTR</td>
</tr>
<tr>
<td>Ensembl transcript ID</td>
<td>ENST00000003084</td>
</tr>
<tr>
<td>Genbank transcript ID</td>
<td>NM_000492</td>
</tr>
<tr>
<td>UniProt peptide</td>
<td>P13668</td>
</tr>
<tr>
<td>alteration type</td>
<td>single base exchange</td>
</tr>
<tr>
<td>alteration region</td>
<td>CDS</td>
</tr>
<tr>
<td>DNA changes</td>
<td>c.3454G&gt;C</td>
</tr>
<tr>
<td></td>
<td>cDNA-5688G&gt;C</td>
</tr>
<tr>
<td></td>
<td>p.148916G&gt;C</td>
</tr>
<tr>
<td>AA changes</td>
<td>D1152H Score: 81 <a href="#">explain scores</a></td>
</tr>
</tbody>
</table>

---

Model: *simple_aae*, prob: 0.999992683327568 (classification due to ClinVar, real probability is shown anyway) [explain](#)
**MUTATION TASTER EXAMPLE – R31C**

**Alteration R31C**

**Prediction**

<table>
<thead>
<tr>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>• amino acid sequence changed</td>
</tr>
<tr>
<td>• heterozygous in TGP or ExAC</td>
</tr>
<tr>
<td>• known disease mutation at this position (HGMD CM931139)</td>
</tr>
<tr>
<td>• protein features (might be) affected</td>
</tr>
</tbody>
</table>

**Analysis of R31C**

<table>
<thead>
<tr>
<th>analysed issue</th>
<th>analysis result</th>
</tr>
</thead>
<tbody>
<tr>
<td>name of alteration</td>
<td>R31C</td>
</tr>
<tr>
<td>alteration (phys. location)</td>
<td>chr7:117144344C&gt;T</td>
</tr>
<tr>
<td>HGNC symbol</td>
<td>CFTR</td>
</tr>
<tr>
<td>Ensembl transcript ID</td>
<td>ENST0000000003084</td>
</tr>
<tr>
<td>Genbank transcript ID</td>
<td>NM_000492</td>
</tr>
<tr>
<td>UniProt peptide</td>
<td>P13569</td>
</tr>
<tr>
<td>alteration type</td>
<td>single base exchange</td>
</tr>
<tr>
<td>alteration region</td>
<td>CDS</td>
</tr>
<tr>
<td>DNA changes</td>
<td>c.81C&gt;T</td>
</tr>
<tr>
<td></td>
<td>cDNA.223C&gt;T</td>
</tr>
<tr>
<td></td>
<td>g.38507C&gt;T</td>
</tr>
<tr>
<td>AA changes</td>
<td>R31C Score: 180 <img src="#" alt="explain score(s)" /></td>
</tr>
<tr>
<td>position(s) of altered AA</td>
<td>31</td>
</tr>
<tr>
<td>if AA alteration in CDS</td>
<td></td>
</tr>
</tbody>
</table>
Evidence of Pathogenicity
- Very strong
- Strong
- Moderate
- Supporting

Evidence of Benign
- Strong
- Supporting

Use combination of criteria to classify sequence variant.
- For example, a variant meeting 1 Very strong and 2 Moderate criteria is classified as Pathogenic
Knowledge accruing daily, however the clinical impact of most variants is unknown.

Lack of data to support a quantitative assignment of variant certainty to any of the five categories – imperfect variant analysis.

Conflicting information from different sources.

Some phenotype are associated with a single gene but many are associated with multiple genes.
Convey patient results effectively to a non-expert physician

- Concise
- Clear
- Easy to understand
- Easy to locate important information
§493.1291 Standard: Test report.

(c) The test report must indicate the following:

- For positive patient identification, either the patient's name and identification number, or a unique patient identifier and identification number.
- The name and address of the laboratory location where the test was performed.
- The test report date.
- The test performed.
- Specimen source, when appropriate.
- The test result and, if applicable, the units of measurement or interpretation, or both.
- Any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability.
Confidential DNA Analysis Laboratory Report

Texas Department of State Health Services
LABORATORY SERVICES SECTION
CLIA #645D0460644
Mailing Address
PO BOX 149347
AUSTIN, TX 78714-9347
1-888-963-7111
www.dshs.state.tx.us

BRACKENRIDGE HOSPITAL
ATTN: LABORATORY
1000 15TH ST
AUSTIN, TX 78750

Date of Report: 02/01/2012
Physician: Smith

Patient: DOE, JOHN
Medical Record: A1234567
Mother: DOE, JANE
Address: 1111 12th ST
Austin, TX 78756

Date of Birth: 01/15/2012
Date of Collection: 01/17/2012
Date Received: 01/28/2012
DNA ID: DV201200082
NBS ID: 20120281102
Specimen: Dried Blood Spot

Test Name: VLCAD DNA Test
Reason for Referral: Possible VLCAD

DNA Test Result: c.652G>A
The results suggest Very Long-Chain Acyl-CoA Dehydrogenase Deficiency. If you have not already done so, consultation with a metabolic specialist within 24 hours is strongly recommended.

Interpretation:
One apparently homozygous variant is identified: c.652G>A

DNA sequencing identified an apparently HOMOZYGOUS PATHOGENIC VARIANT, c.652G>A (E218K), in the very long-chain acyl-CoA dehydrogenase (VLCAD) gene. c.652G>A is a variant at amino acid #218 in exon 8 and results in a change from Glutamate (E) to Lysine (K). This variant has been reported in association with VLCAD deficiency (Andresen BS, et al. 1999b. Am J Hum Genet 64:479-494).

Methodology:
Molecular analysis is performed by automated DNA sequencing of the very long-chain acyl-CoA dehydrogenase (VLCAD) gene. Of the known variants in this gene, approximately 95% can be detected using this methodology. Pathogenic inactivating variants, large deletions/duplications, or whole gene deletions/duplications may not be identified by this methodology. This test was developed and its performance characteristics determined by the Laboratory Services Section at DHS. The test has not been approved or cleared by the US Food and Drug Administration (FDA). Molecular based testing is highly accurate. However, rare diagnostic errors may occur. Test results should not be used as a diagnostic test but should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in the interpretation of results may occur if information given to the laboratory is inaccurate or incomplete.
Reason for testing
Disease locus tested – gene name
Number of sequence variants detected
Name of the variants
    - Genomic DNA level (g.98809_98811delCTT)
    - Complementary DNA level (c.1521_1523delCTT)
    - Protein level (p.Phe508del)
  - Historical naming (ΔF508)
REPORT CONTENT

- Result interpretation
  - Pathogenicity of the variants
  - Associated condition
  - Inheritance pattern
  - Overall NBS result for the condition
  - Reference or literature

- Recommendations
  - Genetic counseling
  - Referral or Request Repeat
  - Supplemental clinical testing
  - Notify family
## VLCAD RESULT RECOMMENDATIONS

### Clinical Significance Statements

1. **The results suggest Very Long-Chain Acyl-CoA Dehydrogenase Deficiency.** If you have not already done so, consultation with a metabolic specialist within **24 hours** is strongly recommended.

2. **If you have not already done so,** consultation with a metabolic specialist within **24 hours** is recommended.

3. **If clinically indicated,** consultation with a metabolic specialist within **72 hours** is recommended. As appropriate, recommend genetic counseling within **30 days.**

4. **Notify family of test results.** As appropriate, recommend genetic counseling within **30 days.**

5. **No clinical significance statement applied.**

### Variants identified

<table>
<thead>
<tr>
<th>Variants identified</th>
<th>Pathogenic</th>
<th>Likely Pathogenic</th>
<th>Variant of Unknown Significance</th>
<th>Likely Benign</th>
<th>Benign</th>
<th>No variant found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Likely Pathogenic</td>
<td></td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Variant of Unknown Significance</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Likely Benign</td>
<td></td>
<td>3</td>
<td></td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No variant found</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Methodology

Limitation
  ▪ Type of variants not detected
  ▪ Detection rate
  ▪ Residual risk of being a carrier for a pathogenic variant not tested for

Disclaimer
Molecular analysis is performed by bidirectional Sanger DNA sequencing of the very long-chain acyl-CoA dehydrogenase (VLCAD) gene. Of the known variants in this gene, approximately 95% can be detected using this methodology. Pathogenic intronic variants, large deletions/duplications, or whole gene deletions/duplications may not be identified by this methodology. This test was developed and its performance characteristics determined by the Laboratory Services Section at DSHS. The test has not been approved or cleared by the US Food and Drug Administration (FDA). Molecular based testing is highly accurate. However, rare diagnostic errors may occur. Test results should not be used as a diagnostic test but should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in the interpretation of results may occur if information given to the laboratory is inaccurate or incomplete.
REPORTING TIMEFRAME

- Which report?
  - Regular newborn screening report
  - Preliminary + Final reports
  - Separate supplemental report

- Timeliness
- Critical results
- Use of a reference laboratory
CLIA Requirement
- Pertinent updates on testing information must be provided to clients whenever changes occur that affect the test results or interpretation of test results.

Set clear policies on reanalysis of genetic testing data
- What can the providers or referring laboratories expect?
- How often?
- How soon?
- For how long?

Set procedures and identify mechanisms and resources to recontact previous users and make a good-faith effort to provide updates and revisions to previous test reports
Electronic reporting (HL7 messaging)
Data submission to variant database such as ClinVar
Exome or Whole genome sequencing
  ▪ Variants in a gene of uncertain significance
  ▪ Incidental findings
THANK YOU!