Kendra Waller[1], Jade Tinker[1], Elaine Chen[1], Ira Lu[1], Tami Johnston[1], Melissa Parra[1], Tim Vo[1], Chia-ling Gau[1]
1) Ambry Genetics Corporation, Aliso Viejo, CA

Reclassification of Historical Mutations in the *CFTR* Gene for Cystic Fibrosis Reveals That 37% of Previously-Classified "Mutations" are Variants of Unknown Significance or Benign Alterations

BACKGROUND

- Pathogenic alterations in the *CFTR* gene have been found to cause classic cystic fibrosis and *CFTR*-related disorders.
- With the advent of full gene sequencing, numerous variants of unknown significance have been identified.
- The American College of Medical Genetics (ACMG) recommends a new 5-tier model for the classification of these variants(1).
- In line with the ACMG guidelines, we have developed a classification scheme to determine the pathogenicity of *CFTR* alterations:
  - 1) Pathogenic Mutation
  - 2) Variant, Likely Pathogenic
  - 3) Variant of Unknown Significance
  - 4) Variant, Likely Benign
  - 5) Benign Alteration
- Classification within each of these categories is contingent upon fulfillment of criteria within the classification scheme (see table).

METHODS

- Since March of 2011, a thorough review of detected alterations classified as "mutations" has been conducted on samples received at our laboratory using a new classification scheme (see table).
- A total of 277 mutations were evaluated with this scheme.
- Alterations in the *CFTR* gene were detected from genomic DNA isolated from the patient's specimen using a standardized kit.
- Following isolation, the DNA was analyzed with either traditional Sanger dideoxy terminator DNA sequencing or next-generation sequencing.

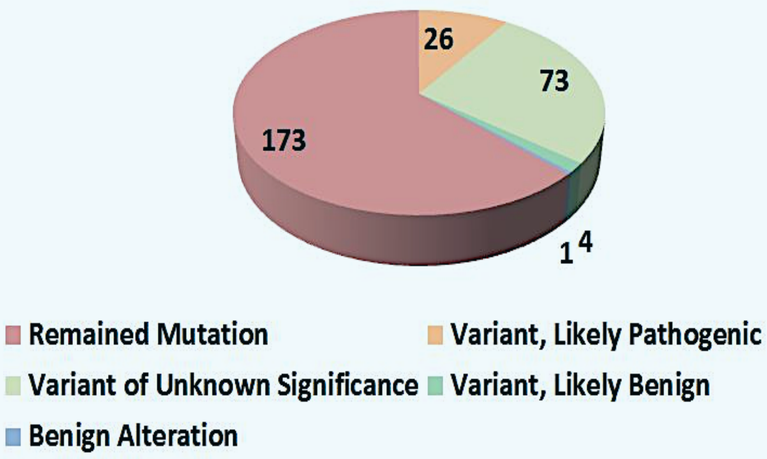
RESULTS

- After analysis, 37.5% (104/277) of alterations previously classified as mutations were reclassified to variants or benign.
- The majority of these alterations were reclassified from mutation to a variant of unknown significance (73/104).
- Of these, 16 historical mutations were reclassified due to conflicting evidence, 45 had insufficient data to classify as a mutation, and 12 did not meet any criteria.
- Twenty-six historical mutations were reclassified to likely pathogenic variants and four more were reclassified to likely benign variants.
- Of note, one historical mutation was reclassified to a benign alteration.

CFTR Classification Scheme (1-11)

CLASS	AMBRY CLASSIFICATION	CATEGORY	CRITERIA
5	PATHOGENIC MUTATION	A 1 NEEDED	ALTERATIONS RESULTING IN PREMATURE TRUNCATION (E.G.READING FRAME SHIFT, NONSENSE)
			OTHER ACMG-DEFINED MUTATION (I.E. INITIATION CODON OR GROSS DELETION)
			FUNCTIONALLY-VALIDATED SPLICING MUTATION
			STRONG SEGREGATION WITH DISEASE (LOD >3 = >10 MEIOSES)
			CONFIRMED *DE NOVO* ALTERATION IN *TRANS* WITH 2ND MUTATION IN INDIVIDUAL WITH CLASSIC DISEASE.
		B 3 NEEDED	DEFICIENT PROTEIN FUNCTION BY *IN VITRO/EX VIVO* ASSAY
			DETECTED IN INDIVIDUAL(S) SATISFYING ESTABLISHED DIAGNOSTIC CRITERIA FOR CLASSIC DISEASE IN *TRANS* WITH A MUTATION OR MUTATION IS HOMOZYGOUS
			LAST NUCLEOTIDE OF EXON
			WELL-CHARACTERIZED MUTATION AT SAME POSITION
			OTHER STRONG DATA SUPPORTING PATHOGENIC CLASSIFICATION
			GOOD SEGREGATION WITH DISEASE (LOD 1.5-3 = 5-9 MEIOSES)
			2 OF CLASSIFICATION OF C (BELOW) MET
4	VARIANT, LIKELY PATHOGENIC	1 NEEDED	ALTERATIONS AT THE CANONICAL DONOR/ACCEPTOR SITES (+/- 1, 2) WITHOUT SPLICING ASSAY DATA IN SUPPORT OF PATHOGENICITY
		C 3 NEEDED	RARE (0.1%) IN GENERAL POPULATION DATABASES (DBSNP, ESP, 1000 GENOMES)
			IN SILICO MODELS IN AGREEMENT (DELETERIOUS) AND/OR [COMPLETELY CONSERVED POSITION IN APPROPRIATE SPECIES AND IN IMPORTANT FUNCTIONAL DOMAIN]
			OTHER DATA SUPPORTING PATHOGENIC CLASSIFICATION
			MODERATE SEGREGATION WITH DISEASE (AT LEAST 3 INFORMATIVE MEIOSES)
		2 OF B	
3	VUS	INSUFFICIENT OR CONFLICTING EVIDENCE	
		GROSS DUPLICATIONS WITHOUT STRONG EVIDENCE FOR PATHOGENIC OR BENIGN	
2	VARIANT, LIKELY BENIGN	D/E 2 NEEDED	INTACT PROTEIN FUNCTION OBSERVED BY *IN VITRO/EX VIVO* ASSAYS
			INTRONIC ALTERATION WITH NO SPLICING IMPACT BY RT-PCR ANALYSIS OR OTHER SPLICING ASSAY
			SYNONYMOUS ALTERATIONS WITH INSUFFICIENT EVIDENCE TO CLASSIFY AS BENIGN
			SEEN IN CONJUNCTION WITH TWO DELETERIOUS MUTATIONS CONFIRMED IN *TRANS* IN SYMPTOMATIC INDIVIDUALS
			IN SILICO MODELS IN AGREEMENT (BENIGN) AND/OR [NOT CONSERVED POSITION IN APPROPRIATE SPECIES AND NOT IN IMPORTANT FUNCTIONAL DOMAIN]
			DOES NOT SEGREGATE IN FAMILY STUDY
			OTHER DATA SUPPORTING BENIGN CLASSIFICATION
1	BENIGN	F 1 NEEDED	ANY SUB-POPULATION FREQUENCY (EXCEPT ASIAN POPULATION) IS >= 0.5%. AN ASIAN SUBPOPULATION MINOR ALLELE FREQUENCY OF >= 0.3% (AT LEAST 3 ALLELES OBSERVED)
			TOTAL INTERNAL FREQUENCY IS >=1.3%
		4 OR MORE OF D/E	

After Analysis (n=277)



TAKE-HOME POINTS

- Effective criteria for the purposes of determining pathogenicity should include functional data, co-occurrence with other pathogenic mutations, population frequency, co-segregation with disease, internal data, and *in silico* models.
- Given the introduction of a new classification scheme in the community, along with an ACMG working group for variant classification standards, many historical mutations are likely to be reclassified and will impact genetic counseling and management for families that harbor these alterations.

REFERENCES

1. Richards et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med* 2008;10:294.
2. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from:www.ncbi.nlm.nih.gov/SNP.Accessed Jan 2012).
3. ESEfinder [Internet]. Smith PJ, *et al.* (2006) *Hum Mol Genet* 15(16):2490-2508 and Cartegni L, etal *Nucleic Acid Research* 2003;31(13): 3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
4. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
5. Grantham R. Amino acid difference formula to help explain protein evolution. *Science* 1974;185(4151):862-864.
6. HGMD® [Internet]: Stenson PD etal. *Genome Med.* 2009;1(1):13 www.hgmd.cf.ac.uk.
7. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org
8. PolyPhen [Internet]: Adzhubei IA, etal. *Nat Methods* . 2010;7(4):248-249. genetics.bwh.harvard.edu/pph2.
9. The 1000 Genomes Project Consortium.An integrated map of genetic variation from 1092 human genomes.*Nature.* 2012;491:56-65.
10. Berkeley Drosophila Genome Project [Internet]. Reese MG etal. *J Comp Biol* . 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
11.SIFT [Internet]: Ng PC & Henikoff S. *Hum Genet.* 2006;7:61-80. http://sift.jcvi.org