



# CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG)

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Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with this technical standard. They also are advised to take notice of the date any particular technical standard was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Pathogenic variants in the *CFTR* gene are causative of classic cystic fibrosis (CF) as well as some nonclassic CF phenotypes. In 2001, CF became the first target of pan-ethnic universal carrier screening by molecular methods. The American College of Medical Genetics and Genomics (ACMG) recommended a core panel of 23 disease-causing variants as the minimal set to be included in pan-ethnic carrier screening of individuals with no family history of the disease, and these variants were usually assessed using targeted methods. The original recommendation also left open the option for laboratories to offer expanded *CFTR* variant panels; however, at the time, expanded *CFTR* variant

panels were met with some controversy on the basis of the available technologies and the limited phenotypic knowledge of rare variants. Both of those aspects have now evolved, prompting this update of the ACMG technical standards for *CFTR* variant testing.

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## INTRODUCTION

It has now been 30 years since the discovery of the *CFTR* gene. Pathogenic variants in this gene are causative of classic cystic fibrosis (CF) as well as nonclassic CF phenotypes, including isolated congenital absence of the vas deferens (CAVD).<sup>1–3</sup> Identification of the *CFTR* gene resulted in the availability of diagnostic testing for symptomatic individuals and carrier testing for at-risk relatives. *CFTR* variant testing is also now offered to reproductive couples in the United States and some other countries for screening purposes, is included in most newborn screening programs, and is being used for personalized therapy.

In 2001, upon joint recommendation by the American College of Medical Genetics and Genomics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG), CF became the first target of pan-ethnic universal carrier screening by molecular methods. Because molecular genetic testing at the time was performed largely by targeted allele-specific assays, the ACMG recommended a core panel of 25 disease-causing variants with an allele frequency of  $\geq 0.1\%$  in the general US population to represent the minimal set to be included in pan-ethnic carrier screening of individuals with no family history of the disease.<sup>4</sup> Aside from the elimination of two variants from the panel in 2004—one because it was much

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rarer than previously thought, and the other because it was determined to actually be a benign variant—this core panel (often referred to as the “ACMG-23”) has remained unchanged since, even as molecular diagnostic technologies and genetic knowledge have dramatically advanced.<sup>5</sup>

However, with the advent of next-generation sequencing (NGS), it has become much easier and less expensive to more extensively interrogate genes including all possible coding variants as well as deep-intronic and other noncoding variants if desired. Furthermore, our knowledge of additional *CFTR* variants at the functional level has invited a broadening of the scope of variation that could potentially be tested for, in either the carrier or diagnostic setting. The original ACMG-ACOG recommendation left open, but did not encourage, the option for laboratories to offer an expanded *CFTR* variant panel beyond the core panel, and at the time, expanded variant panels were met with some controversy on the basis of the available technologies and limited phenotypic knowledge of rare variants. However, both of those aspects have now evolved, prompting this update of the ACMG technical standards for *CFTR* variant testing.

## BACKGROUND ON THE *CFTR* GENE

### Gene symbol/chromosome locus

The *CFTR* (cystic fibrosis transmembrane conductance regulator) gene is located on chromosome 7q31.2 and contains 27 coding exons. The reference messenger RNA (mRNA) and protein sequences are NM\_000492 and NP\_000483, respectively.

### MIM numbers

The MIM number for the *CFTR* gene is 602421, and variants within the *CFTR* gene are associated with autosomal recessive cystic fibrosis (MIM 219700). Variants within the *CFTR* gene are also associated with nonclassic CF phenotypes including autosomal recessive CAVD (MIM 277180), autosomal dominant idiopathic pancreatitis (MIM 167800), and autosomal dominant bronchiectasis (as a modifier) (MIM 211400).

### Brief clinical description of the *CFTR*-associated phenotypes

Classic cystic fibrosis is one of the most common autosomal recessive conditions in Caucasians with a prevalence of approximately 1 in 2500 to 3300 live births. Classic CF is characterized by viscous mucus in the lungs along with involvement of the digestive system, reproductive system, and sweat glands. The degree of pulmonary disease determines the prognosis; recurrent and persistent pulmonary infections are common and lead to respiratory failure.

Pancreatic insufficiency occurs in cases of classic CF, and neonatal meconium ileus occurs in 15% to 20% of newborns with this form of CF. Other findings may include poor growth, poor weight gain despite normal food intake, salty skin, chronic sinusitis, nasal polyps, liver disease, pancreatitis, and CAVD. The overall average survival of classic CF patients, including those with a milder presentation, is approximately 40 years.

Nonclassic CF phenotypes include infertility, idiopathic pancreatitis, bronchiectasis, and chronic rhinosinusitis. The presence of pancreatic exocrine function and intermediate (<90 mmol/l) or normal (<40 mmol/l) chloride levels after employing sweat testing help to distinguish nonclassic from classic forms.<sup>6,7</sup>

### *CFTR* gene description/normal gene product

The *CFTR* gene encodes an adenosine triphosphate (ATP)-binding cassette transporter protein. This protein functions as a channel that transports negatively charged chloride ions across the membranes of various cells that produce mucus, sweat, saliva, tears, and digestive enzymes. The transport of these ions helps control the flow of water in cells, so that the necessary mucus is made, which is used to lubricate the lining of several organs and tissues, including the lungs, digestive system, and reproductive system. The *CFTR* protein also influences the transport of positively charged sodium ions across cell membranes.

### Disease mechanisms/abnormal gene product/treatment

Pathogenic variants in the *CFTR* gene alter the production, structure, or stability of the chloride channel. This impairs the transport of chloride ions and the movement of water into and out of cells, which results in the production of mucus that is abnormally thick and viscous by the cells lining the passageways of the lungs, pancreas, and other organs. Lung disease results from the clogging of the airways due to mucus build-up, decreased mucociliary clearance, and the resulting infection and inflammation. Thickened secretions from the pancreas block the exocrine movement of the digestive enzymes into the duodenum and result in irreversible damage to the pancreas, often with pancreatitis. The lack of digestive enzymes leads to malabsorption, which contributes to malnutrition and poor growth and development. Thickened secretions may also cause liver problems, which can lead to scarring and cirrhosis.

Symptomatic treatment for classic CF includes control of infections, clearance of mucus in the lung, and improvement of nutrition through pancreatic enzymatic replacement. The development of small molecule drugs that target the folding and functional defects associated with abnormal forms of *CFTR* have revolutionized the treatment of the disorder,<sup>8</sup> and many individuals with classic CF will likely benefit from combinations of these newly developed *CFTR*-targeted drugs.<sup>9,10</sup> Patients with nonclassic CF will present with variable phenotypes and disease severity (expressivity). Management of these patients will depend on disease manifestation and severity and are more likely to target the organ system involved (e.g., antibiotic therapy).

### Indications for *CFTR* variant testing

*CFTR* variant testing is used postnatally for diagnosis and in adults for diagnosis and carrier testing. Diagnostic testing can be used for the molecular confirmation of a clinical CF diagnosis, for infants with meconium ileus, for males with

CAVD, for individuals with idiopathic pancreatitis or bronchiectasis, and as a follow-up to newborn screening. Patients with classic CF are more likely to have two variants that together result in a complete loss of protein function, whereas those with nonclassic CF are more likely to have two variants that together result in only a partial loss of protein function. Carriers of a single pathogenic or likely pathogenic variant are expected to be asymptomatic, and couples can be tested sequentially (with the female typically tested first and the male tested only if the female carries a variant) or simultaneously. During pregnancy, simultaneous testing may be desired depending on gestational age, family and personal history, ethnicity, or patient preferences. Carrier testing may be offered to individuals with a positive family history of CF, in partners of individuals with a positive family history, in partners of CAVD males, to reproductive age women, and to gamete donors.

*CFTR* variant testing can also be performed for prenatal diagnosis using cells obtained for diagnostic cytogenetic testing (i.e., amniocentesis or chorionic villus sampling [CVS]). Testing can take place on cultured or uncultured amniocytes or villous trophoblasts.

## TESTING CONSIDERATIONS (WHAT TO TEST)

### Modernizing the ACMG-23 variant panel

The development of the ACMG-23 variant panel followed a careful analysis and revision of the original ACMG-25 variant panel, which was a product of two National Institutes of Health (NIH) consensus conferences (1997 and 1998), followed by a Steering Committee made up of ACMG and ACOG representatives.<sup>4,5</sup> This was the first time professional organizations recommended population-based screening at the DNA level for a genetic condition.

However, along with advances in technology, the past two decades have brought about an improved understanding of genetics and genomics. As a result, (1) the system of variant classification has been refined, (2) the phenotypes associated with CF (both classic and nonclassic forms) have been better characterized, (3) the associations of *CFTR* variants with clinically relevant nonclassic CF phenotypes are now recognized, (4) in vitro genotype–phenotype functional variant analysis exists, and (5) pan-ethnic screening with minimal variation in implementation is accepted.

Expanded carrier screening by NGS now makes it possible to screen for clinically relevant variants without regard to ethnicity.<sup>11</sup> The bottleneck is no longer the number of detectable variants but instead an improved understanding of genotype–phenotype correlation.

### Classification-based (targeted) testing vs. classification-based reporting

As a way to ensure that *CFTR* variant testing for carrier screening and diagnostic testing purposes remains comprehensive, pan-ethnic, and up-to-date, the ACMG recommends either a classification-based reporting approach or a classification-based (targeted) testing approach (which has

historically been used for *CFTR* carrier screening). For those laboratories who wish to continue using a targeted testing approach, the ACMG-23 variant panel remains as the minimum list of *CFTR* variants that should be included. Laboratories may want to consider adding additional variants to their panel depending on the ethnic composition of their expected test population.<sup>12</sup> However, the minimum list of *CFTR* variants recommended for pan-ethnic carrier screening has not been increased at this time.

In contrast, the classification-based reporting approach is based on providing individuals with comprehensive CF testing that minimally includes an assessment of all of the exonic coding regions and  $\pm 2$  bp proximal splice junctions of the *CFTR* gene, followed by the reporting of all pathogenic and likely pathogenic variants for classic CF. Variants of uncertain clinical significance (VUS) are discussed in more detail in “Considerations for variants of uncertain clinical significance.” Variants that are benign or likely benign should not be reported. Targeted and comprehensive approaches are both acceptable for the testing of individuals regardless of race, ethnicity, or test indication.

The classification-based reporting approach is similar to the approach recommended for the reporting of secondary findings in the setting of genomic sequencing (i.e., exome and genome sequencing), whereby specific variant classifications/types in specific genes are recommended for reporting but specific variants are not recommended.<sup>13,14</sup> Many laboratories also use a similar approach for the reporting of primary findings from diagnostic genomic sequencing, with pathogenic and likely pathogenic variants being reported. Further considerations on specific aspects of these approaches for *CFTR* testing are provided below.

### Considerations for intronic variants

Aside from the important polyT/TG region in intron 9 (discussed in more detail in “Considerations for the intron 9 polyT and TG regions”) and the c.2657+5G>A (also known as 2789+5G>A) and c.3718–2477C>T (also known as 3849+10kbC>T) variants included in the original ACMG-23 variant panel, little attention thus far has been paid to deep-intronic variants in the *CFTR* gene, at least at the clinical level. Targeted variant testing would not typically interrogate such variants, nor would exome sequencing (other than the detection of potential splice variants surrounding the proximal intron–exon junctions). However, with genome sequencing likely to become more common in clinical molecular diagnostics in the coming years, it is inevitable that deep-intronic variants in *CFTR* will be increasingly observed and will need to be annotated. In most cases, the question to be answered will be whether the intronic variant creates a cryptic splice site leading to inclusion of a pseudoexon or loss of a true exon in the mRNA product of the gene, resulting in an abnormal CFTR protein.<sup>15</sup> Despite the existence of splice variant prediction algorithms, the best confirmatory evidence would be obtained with RNA sequencing, but that approach may require the biopsy of a tissue in

which *CFTR* is sufficiently expressed, and such invasive studies would not be practical in most clinical laboratory settings.<sup>16</sup> In the absence of RNA studies, it is recommended that laboratories exercise caution in classifying and reporting deep-intronic variants, if detected.

As they were originally part of the ACMG-23 carrier screening variant panel, for all prenatal, postnatal, and adult diagnostic testing and carrier screening indications for *CFTR* sequencing, the ACMG recommends the reporting of the c.2657+5G>A and c.3718–2477C>T intronic variants. If these variants are not detectable with the laboratory's methodology (e.g., exome sequencing), then a separate assay should be performed to test for these variants.

### Considerations for deletions/duplications

Gene deletions/duplications may include a single exon, multiple exons, or the entire gene. In addition, some deletions may extend beyond the entire gene and have additional implications. While deletions and duplications occur in *CFTR*, their frequency is estimated at <5% of all detected *CFTR* variants, which may be an underestimate due to a historical lack of copy-number variant (CNV) data available from standard analyses.

For all prenatal, postnatal, and adult diagnostic testing and carrier screening indications for *CFTR* testing, the ACMG does not recommend the testing of any specific exon-level or gene-level deletion or duplication variants. However, as larger deletions and duplications are highly likely to be pathogenic if detected, the ACMG recommends that laboratories using classification-based reporting methods should be capable of evaluating for the presence of deletions and duplications in prenatal and postnatal diagnostic indications, especially when only a single variant is initially detected. If a laboratory does not currently have the capability to detect deletions and duplications using their primary testing methodology, it is recommended that laboratories validate their ability to detect deletions and duplications using an alternate methodology or assist in arranging for suitable send-out testing to be ordered by clinicians when needed.

## METHODOLOGY CONSIDERATIONS (HOW TO TEST)

All general recommendations described in the ACMG Technical Standards for Clinical Genetics Laboratories apply. The following additional details are specific for *CFTR* variant testing.

### Positive controls

Positive controls for many *CFTR* variants can be obtained from the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository as either cell lines or DNA. Synthetic super controls that include multiple variants are commercially available.

### Sample preparation

*CFTR* variant testing typically occurs using genomic DNA prepared from whole blood using a variety of automated and

manual extraction methods. Other extraction methods can also be used that accommodate buccal samples (e.g., brushes, swabs), saliva, and prenatal specimens (e.g., amniocytes, chorionic villi).

### Method verification/validation

For *CFTR* variant analysis, laboratories can choose between the creation of a laboratory developed test (LDT) or the use of an existing in vitro diagnostic (IVD) (i.e., an FDA-cleared or approved test). Laboratories offering genetic tests for clinical purposes are regulated under CLIA. IVD tests require a method verification by the performing laboratory, whereas LDTs require a more comprehensive method validation.

### Targeted vs. comprehensive methods

Historically, a variety of molecular techniques were used to identify various types and numbers of *CFTR* variants, and the identification of *CFTR* variants could be broadly divided into two categories: (1) methods that targeted the detection of known variants, and (2) more comprehensive methods that attempted to detect all variants without a need for any prior knowledge regarding the identity or precise location of any particular variant. The specific testing strategy utilized by a laboratory often involved one or a combination of both types of methods (e.g., reflex testing), and laboratories were aware of the limitations of the method(s) chosen.

The ACMG recommends that laboratories performing initial *CFTR* variant testing on an individual can use either targeted or comprehensive methods to evaluate the gene. The testing methodology chosen by a laboratory for a specific indication, including the corresponding limitations, should be clearly communicated in the report. If pathogenic or likely pathogenic *CFTR* variants have been confirmed in *both* biological parents, or an affected full sibling, only targeted methods should be used. Several targeted methods are described in detail in the ACMG Technical Standards for Clinical Genetics Laboratories. Information regarding some comprehensive methods for CF testing are described further below.

### Comprehensive methods for the detection of *CFTR* variants

#### *Sanger sequencing*

Sanger sequencing has been routinely used in clinical laboratories for the analysis of *CFTR* due to its robustness, accuracy, and ease of set-up. Sanger sequencing protocols for the analysis of *CFTR*, including the 5'-flanking region, 27 coding exons, and selected intronic regions, have previously been published.<sup>17</sup> Sanger sequencing can also be used for targeted testing of specific familial variants that have previously been identified. However, some important limitations related to the Sanger sequencing of *CFTR* include low throughput, the possibility of allele dropout and false-negative results, and an inability to detect large deletions or duplications.

#### *Next-generation sequencing*

NGS technologies have revolutionized the genetic testing field with a substantial reduction in cost per base and a

considerable enhancement of sequencing capacities, allowing for the simultaneous analysis of single genes or panels of selected genes in a large number of patients at a previously unprecedented speed and cost.<sup>18</sup> NGS-based protocols for the analysis of *CFTR* have demonstrated acceptable results; however, several drawbacks have remained, such as a risk of false-negative or false-positive results and the challenge of sequencing specific regions (often requiring additional Sanger sequencing to adequately assess those regions).

Deletions and duplications can also be analyzed from NGS data using normalized depth of coverage and paired-end mapping, and a workflow for *CFTR* variant analysis using NGS for the simultaneous detection of single-nucleotide variants (SNVs) and large rearrangements has also previously been published.<sup>19</sup> While NGS is currently more time- and cost-effective than running a separate companion assay for dosage analysis, the sensitivity and specificity of the bioinformatics analysis for large deletions and duplications depends highly on the quality of NGS data produced.

#### *Multiplex ligation-dependent probe amplification (for deletion/duplication testing)*

Multiplex ligation-dependent probe amplification (MLPA) is a polymerase chain reaction (PCR)-based method for quantifying multiple genomic loci in a single reaction. It is based on the ligation of a set of two oligonucleotide probes that have annealed adjacently to a target sequence, and only ligated probes can serve as a template for a subsequent PCR. MLPA is an efficient method to detect large deletions and duplications in the *CFTR* gene, and commercial reagents exist.<sup>20</sup> A limitation of MLPA is that any DNA sequence variants located directly under probe binding sites may interfere with probe hybridization and could result in false-positive carrier (one copy) or diagnostic (zero copies) results.

#### *Exon-targeted array CGH (for deletion/duplication testing)*

High-resolution array comparative genomic hybridization (aCGH) with oligonucleotide probes densely distributed across individual genes can be developed to detect copy-number changes at the single-exon level. Oligonucleotide probes can be designed in silico for any sequenced region of a genome, thus allowing the flexibility of interrogating any region of interest. An exon-targeted aCGH for the detection of intragenic *CFTR* CNVs has been described.<sup>21</sup> One advantage of aCGH over MLPA (or other PCR-based methods) is that whenever a deletion or duplication event is identified, its breakpoints can be narrowed down to more precise genomic locations. This greatly facilitates the effort of breakpoint characterization at the DNA sequence level. Furthermore, the high probe density across the gene serves to avoid the pitfalls of false-positive single-exon losses that can result from DNA sequence variants affecting primer or probe binding sites.

## REPORTING CONSIDERATIONS (WHAT TO REPORT)

### Considerations for variant nomenclature/classification

The use of standard Human Genome Variation Society (HGVS) nomenclature is required for the accurate communication of genetic testing results to health-care providers.<sup>22</sup> However, for conditions such as CF, laboratory geneticists and clinicians may still wish to use historical, common terminology (such as deltaF508) in their reports and clinical notes, and it is currently acceptable to describe a *CFTR* variant using historical nomenclature as long as standard HGVS nomenclature is used as well.

Regardless of the test indication, all *CFTR* variants should be classified using ACMG sequence variant classification recommendations.<sup>23</sup> Information from *CFTR* variant-containing databases can be used to inform those variant classifications.

### Considerations regarding the use of *CFTR* variant-containing databases

#### *CFTR2*

The Clinical and Functional Translation of *CFTR* (*CFTR2*) project was initiated in 2008 to expand the clinical annotation of *CFTR* variants beyond the original ACMG-23 variant panel. However, the utility of genetic testing for the diagnosis of, and therapy for, CF has expanded the goal of the *CFTR2* project to encompass all variants reported in *CFTR*.<sup>24</sup> Of ~2000 variants reported in the CF Mutation Database ([www.genet.sickkids.on.ca](http://www.genet.sickkids.on.ca)), >1600 occur in the >89,000 subjects participating in the *CFTR2* project.

Annotation of *CFTR* variants by the *CFTR2* team is performed using a three-level approach: clinical criteria, functional assessment, and penetrance analysis. Once variants have been assessed, results are uploaded to the *CFTR2* website ([CFTR2.org](http://CFTR2.org)) so that interpretations are immediately available to the public. Variant assessments within the *CFTR2* project align well with assignments of disease liability using the ACMG variant classification recommendations.<sup>25</sup> Since the selection of variants for annotation is based on worldwide frequency, rather than country, ethnic, or racial frequencies, variant information in *CFTR2* is applicable to all populations. The pan-ethnic nature of these data enable interpretation of assays that interrogate the entire coding region of *CFTR* in individuals regardless of race or ethnicity.

While it is possible to indicate that certain variants do not cause CF, it does not exclude the possibility that they alter *CFTR* function and contribute to a “*CFTR*-related disorder” (i.e., male infertility, pancreatitis). Furthermore, the *CFTR2* project is unable to assign a small subset of variants (approximately 5%) to disease or non-disease-causing categories due to incongruities among clinical, function, or penetrance data. Some of these variants are classified as variants of varying clinical consequence by the *CFTR2* project, and they have been reported both in individuals with

CF as well as in healthy individuals (in *trans* with a known disease-causing *CFTR* variant in each situation). The variants of varying clinical consequence are often associated with functional thresholds that allow genetic and environmental modifiers to determine whether an individual manifests CF.<sup>25</sup> These observations also help to substantiate reporting VUS (see “Considerations for variants of uncertain clinical significance”).

#### ClinVar

ClinVar ([www.ncbi.nlm.nih.gov/clinvar](http://www.ncbi.nlm.nih.gov/clinvar)) contains *CFTR* variants that have been expertly curated by the CFTR2 project, variants that are generally rare and have been evaluated by clinical molecular diagnostic laboratories, and variants that have been reported by researchers. The level of confidence for the disease annotation assigned to variants outside of the CFTR2 project varies. A small fraction of rare variants have been reported by multiple laboratories and have the same assertion of pathogenicity, lending a degree of confidence to these assignments. However, for other variants, only a single laboratory has provided an assertion or multiple laboratories provided different variant classifications. In most cases, variants reported outside of the CFTR2 project have not had in-depth functional assessment, and in an unknown fraction of cases, the diagnosis of CF has not been confidently confirmed. Therefore, laboratories should exercise caution when evaluating non-CFTR2 assertions for *CFTR* variants within ClinVar.

#### Considerations for the determination of variant phasing (*cis* vs. *trans*, *de novo*)

For many rare recessive disorders, the finding of two potentially pathogenic variants in an affected individual requires the determination of whether they are in *cis* (on the same chromosome) or in *trans* (on different homologous chromosomes) to clarify the diagnosis. Parental testing is typically recommended in this setting to assess the origin of each of the variants so that targeted testing can be offered to other family members who wish to know their carrier status.

For CF, finding two well-described pathogenic variants (those with an allele frequency of  $\geq 0.01\%$  in the CFTR2 database) in an affected individual may not require additional testing to support the diagnosis. However, finding a rare novel *CFTR* variant in combination with a well-established pathogenic *CFTR* variant in an affected individual would ideally result in the ascertainment of both parents to determine the phase and confirm the diagnosis. A rare variant in an affected individual may also be *de novo*, though *de novo* events are rarely observed in individuals with CF.

If it is not possible to obtain samples from both parents and/or additional family members to determine the phase, caution should be used in the interpretation of the results. Laboratories should have a policy for follow-up familial testing, including what types of samples they will accept, and who can provide those samples.

#### Considerations for variants of uncertain clinical significance

As more individuals undergo comprehensive sequencing, the possibility of finding genetic variants with uncertain clinical significance increases. VUS arise from an inability to formally associate a variant and phenotype, which can be due to reduced penetrance, variable expressivity due to modifier genes and/or environmental factors, pleiotropy associated with variants in different regions of the same gene, as well as the possibility that the variant is non-disease-associated and is benign.

More than 2000 variants within *CFTR* have already been identified; however, most of the private or very rare variants are currently classified as VUS. Laboratories routinely reassess VUS to seek a more definitive classification (benign, likely benign, likely pathogenic, pathogenic), and unlike pathogenic and benign variants, the VUS classification is generally not static.<sup>26</sup> Furthermore, genotype–phenotype correlations exist on a spectrum, and age-related penetrance of nonclassic phenotypes is common.

Some concerns surrounding the appropriateness of reporting VUS to health-care providers include unnecessary anxiety for the patient and the possibility for them to make reproductive decisions (e.g., pregnancy termination) based on misinformation. On the other hand, autonomy reflects the right of patients to know, become educated, and make their own decisions.

For all prenatal, postnatal, and adult diagnostic testing indications for *CFTR* where comprehensive methods are used, the ACMG recommends the reporting of VUS. For all adult carrier screening indications for *CFTR* where comprehensive methods are used, VUS should generally not be reported. However, laboratories may want to consider reporting VUS in the partner of an individual who had a pathogenic or likely pathogenic variant detected during screening.

#### Considerations for variants associated with variable expressivity

Some *CFTR* variants are associated with variable expressivity (these are referred to in the CFTR2 database as variants of varying clinical consequence). The phenotypes that may be associated with one of these variants when in *trans* with another one of these variants (or even when in *trans* with a known pathogenic variant) cannot often be predicted, and therefore results involving these variants should be interpreted carefully and reported thoughtfully.

For all prenatal, postnatal, and adult diagnostic testing indications for *CFTR* where comprehensive methods are used, the ACMG recommends the reporting of any variants associated with variable expressivity. In addition to the corresponding ACMG variant classification, if a variant has been classified by the CFTR2 project as a variant of varying clinical consequence, this terminology should also be included in the report. For all adult carrier screening indications for *CFTR* where comprehensive methods are used, variants associated with variable expressivity that are not currently classified as a pathogenic or likely pathogenic variant by the

laboratory should generally not be reported. Laboratories may want to consider reporting variants associated with variable expressivity in the partner of an individual who had a pathogenic or likely pathogenic variant detected during screening.

The specific R117H variant and associated polyT tract is described further below.

### Considerations for the intron 9 polyT and TG regions

The c.350G>A (p.Arg117His, commonly referred to as R117H) *CFTR* variant is associated with variable expressivity, and its consequence is dependent on the status of the polythymidine (polyT) tract in intron 9 (c.1210–12T[5], c.1210–12T[7], or c.1210–12T[9]).

For R117H/5T individuals, additional testing is needed to determine the phase of these variants, though sometimes phase can be inferred for a variant with a well-known polyT haplotype present on the opposite allele. For example, c.1521\_1523delCTT (p.Phe508del) and 9T occur almost completely in *cis*. R117H and 5T in *cis* with a known CF-causing variant on the opposite allele generally results in nonclassic CF. R117H in *cis* with 7T variant and a CF-causing variant in *trans* may have a phenotype varying from asymptomatic to CAVD (in males) and very rarely to nonclassic CF.

Independent of R117H, the 5T allele by itself is associated with variable penetrance for CF and CAVD based on the status of an adjacent poly TG tract, which usually contains 11, 12, or 13 repeats (c.1210–34TG[11], c.1210–34TG[12], c.1210–34TG[13]). When paired with a known CF-causing variant, 5T and 11TG variants in *cis* rarely confer an increased risk for CAVD in males while 5T in *cis* with 12TG or 13TG confers risk for CAVD and rarely for nonclassic CF. Given the commonness of the 5T allele (one in ten individuals carry a 5T variant), interpretation of its disease liability should ideally be performed in the context of the number of associated TG repeats.

For all prenatal, postnatal, and adult diagnostic testing indications for *CFTR*, the ACMG recommends the reporting of R117H status as well as the results from at least the associated polyT tract. For all adult carrier screening indications for *CFTR*, polyT status should be reported when the R117H variant is detected; laboratories may also want to consider reporting the results from the associated polyT tract in the partner of an individual who had a pathogenic or likely pathogenic variant detected during screening.

### ADDITIONAL CONSIDERATIONS FOR PRENATAL DIAGNOSTIC TESTING

When indicated in the prenatal setting:

1. Targeted sequencing for specific *CFTR* variants may be considered when
  - (a) A pathogenic or likely pathogenic variant is confirmed in both partners.
  - (b) A pathogenic or likely pathogenic variant is confirmed in one partner and a VUS or variant

associated with variable expressivity is confirmed in the other partner.

- (c) As part of preimplantation genetic testing when both biological parents are confirmed carriers of a pathogenic or likely pathogenic variant.
2. Comprehensive *CFTR* sequencing may be considered when
    - (a) One member of a couple is known to be a carrier of a pathogenic or likely pathogenic variant and any of the following is also true:
      - i. The partner is unavailable for screening;
      - ii. The partner has not been screened and any of the following is also true:
        1. Screening that partner would be cost prohibitive;
        2. The results from the partner would not be available in time to allow for reproductive decision making;
        3. A diagnostic procedure (e.g., CVS, amniocentesis) is also being performed for other reasons (e.g., ultrasound abnormality).
    - (b) An ultrasound finding (i.e., fetal echogenic bowel) suggests an affected fetus and *CFTR* variant information is not available from either biological parent.

## QUALITY ASSURANCE

### Orthogonal confirmation

Please refer to other ACMG documents regarding general recommendations for the confirmation of germline variants that were originally detected using next-generation sequencing; Sanger sequencing is a commonly used method for this purpose.<sup>18</sup> Orthogonal confirmation is not typically employed when methods other than NGS are used for initial *CFTR* testing. Laboratories should determine during the course of test validation whether orthogonal confirmation is required for the reporting of certain variants and what criteria will be used to make that determination. It is recommended that *CFTR* reports based on NGS methods clearly state whether or not a reported variant was confirmed using an alternate methodology; if orthogonal confirmation is not employed, a brief description of the criteria used to make that determination in the methods section of the report is recommended.

### Variant reevaluation

Existing variant classifications may require modification as new evidence emerges. Please refer to the corresponding ACMG documents on the responsibilities of the laboratory and clinician regarding variant reevaluation.<sup>26,27</sup>

## CONCLUSION

The ACMG recognizes that the detection capabilities of laboratories for SNVs and small insertions and deletions

within the coding regions of genes are no longer a limiting factor for testing. The classification-based reporting approach is already used for diagnostic testing of many conditions, with pathogenic variants, likely pathogenic variants, and some VUS being reported. While these technical standards specifically address *CFTR* and its associated phenotypes, a classification-based reporting approach could eventually also be applied to other genes for carrier screening. However, there may be more VUS present in other genes, as many of the variants detected in other genes may not have been as extensively evaluated as the variants detected in *CFTR*.

In the future, laboratories may want to further consider the potential benefits (and potential negative impacts) of reporting all *CFTR* VUS for carrier screening. The joint interpretive capabilities of clinical laboratories and medical providers are expected to improve over time as more variants are routinely evaluated within the context of specific patient presentations and familial situations, ultimately leading to a greater degree of shared decision making between the patient and provider.

## DISCLOSURE

J.L.D., C.A., D.d.G., W.W.G., K.G.M., and S.R. all serve as directors in clinical laboratories that perform a breadth of genetic and genomic analyses on a fee-for-service basis. K.G.M. is an employee of GeneDx, a wholly owned subsidiary of OPKO Health, Inc. The other authors declare no conflicts of interest.

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