frequently asked questions about

CYSTIC FIBROSIS (CF) TESTING

Is it best to test for as many cystic fibrosis transmembrane regulator (*CFTR*) gene variants as possible when performing carrier testing on healthy individuals?

No. Cystic fibrosis (CF) screening panels should include only a carefully selected list of known pathogenic variants. Although more than 2,000 variants have been identified in the *CFTR* gene, fewer than 200 are known to be causative for CF disease. The majority of *CFTR* variants have little evidence to indicate whether they are benign or disease-causing.

Carrier screening for expectant couples is performed to identify those at risk for having a child with classic CF disease, which is defined by significant pulmonary disease and pancreatic insufficiency.[1] Many *CFTR* variants do not cause classic CF disease. For example, when an individual has a mild CF variant as well as a severe CF variant on the opposite-chromosome, they may have no symptoms or may have a *CFTR*-related disorder, such as pancreatitis, bronchiectasis, or bilateral absence of the vas deferens (BAVD), but the combination does not cause classic CF.

ARUP's 165 CF variant panel includes the 23 variants recommended for screening by the American College of Medical Genetics and Genomics (ACMG), as well as an additional 142 variants known to cause CF disease.[2] Variants of mild or unclear significance were purposely excluded from the panel. That sets ARUP's panel apart from other expanded CF screening panels that often include variants of varying clinical consequences.

If a variant of varying clinical consequence is identified in one member of a couple, this often leads to recommendations for their reproductive partner to undergo screening. If the partner is also positive for a CF variant, the clinical significance to their offspring may be difficult to predict, complicating prenatal testing and decision-making.

The original CF variant panel recommended by the American College of Obstetrics and Gynecology in 2001 included 25 variants. However, after this panel became standard of care, several publications called into question the clinical significance of the I148T variant. By itself, this variant is now known not to be associated with CF disease. Another pathogenic variant, 3199del6, rarely found on the same chromosome as I148T, was determined to be the actual pathogenic variant. This was discovered only after several healthy adults undergoing carrier screening were found to have the I148T variant as well as a severe CF variant on the opposite chromosome. Thus, it was determined that the I148T variant was actually not CF-causing.

Unfortunately, many women who screened positive for the I148T variant were informed they carried a CF-causing variant. Only one in 100 of them also carried the true variant, 3199del6. This undoubtedly led to unnecessary testing of reproductive partners and pregnancies, as well as the termination of healthy pregnancies. A similar scenario could easily occur with other rare CF variants or with variants of varying clinical consequence.

Why does the detection rate of CF expanded panels vary between laboratories?

By definition, expanded panels contain variants found at a frequency of less than one in 1,000 in the CF population. Thus, trying to determine the frequency of the rare variants in a specific ethnicity can be very difficult. The CFTR2 database (which has data on over 80,000 CF patients), publishes the overall frequency of each variant but does not collect data on the frequency based on ethnicity. It can be very misleading to determine the frequency of rare variants from small studies that do report data by ethnicity. For example, if 50 Hispanic individuals affected with CF undergo sequencing to determine the causative variants, any single variant identified will appear to have at least a 1% allele frequency compared to identifying the same variant once in the 88,000 individuals which comprise the CFTR2 database. Including the frequency of such rare variants reported in small studies artificially inflates the reported detection rate in various ethnicities.

When should the mild 5T variant be tested?

The 5T variant is a common mild variant occurring in one in ten individuals in the general population.[3] It causes abnormal splicing of the *CFTR* gene transcript, resulting in a 90% reduction of functional CFTR protein. Most individuals who have two copies of the variant are asymptomatic, although some may have a *CFTR*- related disorder, such as BAVD, pancreatitis, or bronchiectasis.

The 5T variant should only be tested when a patient has symptoms of CF or a *CFTR*-related disorder, or when the R117H variant is identified. When the 5T variant is present on one chromosome and another variant is present on the opposite chromosome, it may help explain *CFTR*-related symptoms. The 5T variant contributes only to classic CF disease when accompanied by another pathogenic variant

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located on the same chromosome as well as a severe pathogenic variant on the opposite chromosome; by itself, it is not causative for CF.

The R117H variant by itself is a mild, non CF-causing variant. But when it is present on the same chromosome as the 5T variant, it may result in CF disease when combined with a severe pathogenic variant on the opposite chromosome.

ACMG recommends reporting the 5T variant only in symptomatic individuals or when the R117H variant is detected by the CF panel. Reproductive partners of individuals who carry the 5T variant alone do not need to undergo CF carrier screening. Prenatal diagnosis is not recommended for pregnancies where one individual carries the mild 5T variant and their reproductive partner carries a severe pathogenic variant because the pregnancy is not at increased risk for CF. For the above reasons, the ACMG recommends against routine testing for the 5T variant in healthy individuals undergoing CF carrier testing, as it caused unnecessary anxiety, testing of the individual's reproductive partner, and testing of the pregnancy.

Thus, ARUP only reports the 5T variant in symptomatic individuals or those positive for the R117H variant when the CF 165 Variants assay is ordered.

How does testing of symptomatic individuals differ from screening healthy individuals for CF carrier status?

The CF 165 Variant assay is the recommended test for individuals who either have symptoms of CF or are undergoing reproductive screening.

The identification of one *CFTR* gene variant in a healthy individual confirms carrier status. The detection of two pathogenic *CFTR* gene variants on opposite chromosomes confirms a diagnosis of CF in a symptomatic patient. If two pathogenic variants are not identified in a symptomatic individual, then *CFTR* gene sequencing and deletion/duplication analysis should be considered to exclude 99% of pathogenic variants.

What type of testing is recommended for individuals with symptoms of a *CFTR*-related disorder, such as congenital bilateral absence of the vas deferens (CBAVD), isolated pancreatitis, or nasal polyps?

Up to 80% of men with CBAVD have at least one identifiable *CFTR* variant. Approximately 20% have two *CFTR* variants (usually one severe and one mild); 33% have one copy of the 5T variant and another *CFTR* variant; 20% have only a single *CFTR* variant; and 1-2% have two copies of the 5T variant.

Individuals with isolated pancreatitis or asthma also have a higher proportion of *CFTR* variants than the general population.

Since many mild variants causing *CFTR*-related disorders are not identified by CF panels designed to detect moderate to severe disease-causing variants, *CFTR* gene sequencing and deletion/duplication analysis is recommended for such patients.

Why does the laboratory need to know a patient's ethnicity, symptoms, and whether there is a family history of CF?

Each of these factors affects residual risk present after a negative CF test. If there is a family history of CF, it is important to specify whether the family member is symptomatic or just a carrier, the relationship of the patient to the family member, and the variant(s) present in the family member. This information is necessary for accurate test interpretation and recommendations.

Consider the usefulness of the above information in the following scenarios:

The CF variant panel is requested on a newborn reported to have a full sister affected with CF caused by two copies of the pathogenic F508del variant. A single F508del variant is detected in the newborn using the CF 165 variant panel. Thus, the newborn is predicted to be unaffected with CF and is merely a carrier, since the CF panel is known to test for both of the familial variants.

The CF 165 variant panel is ordered on an expectant woman whose first cousin is affected with CF. Neither the patient's ethnicity nor the specific variants in her cousin are provided. The woman is negative for variants detected by the CF panel. Without knowledge of the specific familial variants, the significance of a negative variant panel is lessened, since it is not known if her cousin's *CFTR* variants are included on the panel. Nevertheless, if her ethnicity is provided, it is still possible to perform a Bayesian calculation and provide a revised risk estimate for the patient using her specific family history and negative test result.

References

1. Committee Opinion No. 486: Update on Carrier Screening for Cystic Fibrosis. *Obstet Gyn.* 2011;117:1028–1031.
2. Watson M, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med.* 2004; 6:387–391.
3. Friedman KJ, et al. Rapid characterization of the variable length polythymidine tract in the cystic fibrosis (*CFTR*) gene: association of the 5T allele with selected *CFTR* mutations and its incidence in atypical sinopulmonary disease. *Hum Mutat.* 1997;10:108–115.
4. Chillon M, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *NEJM.* 1995;332:1475–1480.

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