

EDUCATIONAL
GUIDE

Carrier Screening Results:
*How to Ensure Reliable
Information for All Patients*



Updated information on cystic fibrosis, spinal muscular atrophy,
and fragile X syndrome carrier screening for clinicians

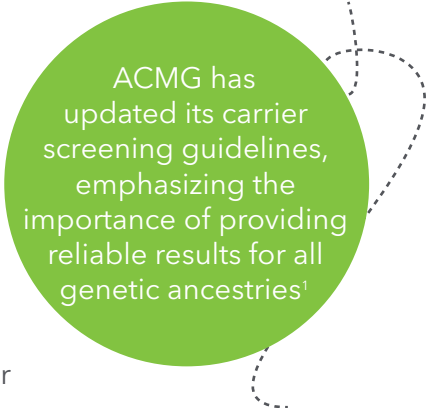
Advanced Genetic Testing Delivers More Reliable Results for Carrier Screening

Three of the conditions most frequently recommended for carrier screening are cystic fibrosis (CF), spinal muscular atrophy (SMA), and fragile X syndrome (FXS). Unfortunately, conventional approaches have tended to deliver uneven results across ancestries, and they often fail to detect more complex genetic variants with important clinical implications.

Recently, the American College of Medical Genetics and Genomics (ACMG) updated its carrier screening guidelines¹. The new recommendations emphasize the importance of adopting methods that produce meaningful results for patients of all ancestries – and of allowing physicians to make consistent carrier screening recommendations for all patients without having to base decisions on ancestry or ethnicity.

Carrier screening is moving to a pan-ancestry model. This transition will offer enormous benefit to patients by ensuring more reliable results for all. However, getting there will require abandoning genetic tests that don't hold up to this new standard, and ensuring that all carrier screening tests utilized are based on insights gleaned from diverse, population-scale data sets. In addition, these tests must be robust enough to reliably detect even the most challenging genetic variants.

How does this affect carrier screening for commonly tested conditions including cystic fibrosis, spinal muscular atrophy and fragile X? Consider these important facts related to carrier screening for each disease:



ACMG has updated its carrier screening guidelines, emphasizing the importance of providing reliable results for all genetic ancestries¹

Cystic fibrosis

- 95% of cystic fibrosis variant information in the “gold-standard” database (CFTR2) is from people of European descent²
- Multiple studies^{3,4} have found the ACMG 23-variant recommendation to be insufficient for detecting cystic fibrosis carriers in diverse populations
- More variants do not guarantee more coverage: larger variant panels still lack sufficient coverage of diverse populations because they were designed based on ethnically homogeneous data^{3,5}
- People of non-European descent are less likely to be detected on prenatal and newborn screening tests even when they are carriers or have cystic fibrosis, due to use of panels lacking coverage for diverse populations^{6,7,8}

Spinal Muscular Atrophy

- If a test is reporting *SMN1* copy number alone for carrier screening, there can be up to approximately 30% false negative results, depending on patient ancestry⁹
- These false-negative results are due to “silent carriers” that have 2 copies of the *SMN1* gene, but both copies of the gene are on the same allele. Thus, a “silent carrier” can still pass on an allele with no copies of *SMN1* in this case. If both parents pass on alleles with no copies of *SMN1*, the child will have SMA.
- This “silent carrier” phenomenon is more common in people of African descent⁹, meaning determining the risk of “silent carrier” genotype may be very important in this demographic

Fragile X Syndrome

- To determine the risk of a potential carrier having a child with fragile X, triplet repeats (CGGs) in the *FMR1* gene must be quantified. However, it is difficult for many technologies to quantify these repeats accurately.
- In addition, there are triplet-repeat interrupting sequences (AGGs) that can significantly alter the risk of certain women for having a child with Fragile X^{4,10,11}. For example, quantifying AGG interruptions adjusts the risk for 90% of certain female carriers compared to CGG repeats alone⁴, but clinicians may not be receiving this information for their patients.

Fortunately, the public release of data from diverse large-scale genomic studies has now made it possible to identify relevant genetic alterations across populations. By incorporating this information into carrier screening tests, and by building these tests with advanced technology capable of detecting all relevant genetic information regardless of complexity, modern carrier screening can deliver reliable results for all patients.

Cystic Fibrosis Carrier Screening

Underrepresented genetic diversity in data sets is a common challenge in genomics today. The effects of this can be seen in diagnostic and carrier screening tools that provide more accurate and comprehensive results for some populations than for others.

Consider these metrics about available genomic data sets: In 2009, an analysis of genome-wide association studies found that 96% of the 1.7 million samples reviewed came from individuals of European ancestry¹². By 2021, data from the GWAS Diversity Monitor website¹³ indicated that the share of GWAS samples from people with non-European ancestry had grown from that measly 4% to just 12%.

The vast majority of historically available data supporting cystic fibrosis testing also comes from people of European descent. The prominent CFTR2 database² includes data from nearly 90,000 patients on pathogenic variants detected in the *CFTR* gene, connecting patient genotype and phenotype. It has long been considered the gold standard on genetic data for cystic fibrosis. Unfortunately, of the patients represented in the database, 95% have European ancestry, leaving other populations woefully underrepresented (Figure 1).

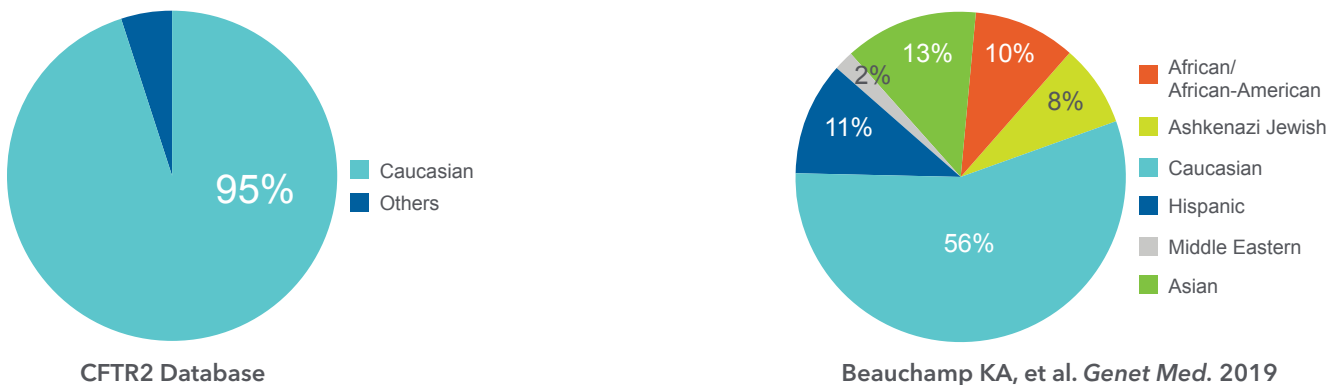


Figure 1. Illustration of the CFTR2 variant database² patient ethnicity compared to more realistic representation of U.S. population diversity³.

To add to the challenges in *CFTR* testing, there have been over 2000 *CFTR* mutations documented¹⁴, with varying levels of pathogenicity. In order to provide practical cystic fibrosis carrier screening guidance for clinical laboratories, ACMG recommended testing for 23 of the most common mutations in 2004, based on available data at that time¹⁵.

According to a study of >370,000 people with an ethnic distribution representative of the U.S. population⁴, **44% of CF carriers would be missed using the ACMG-23 panel**

Since then, several large-scale studies have reported the lack of coverage for diverse populations by the ACMG 23 recommended variants. One study of over 370,000 people with an ethnic distribution representative of the U.S. population found that 44% of carriers would have been missed using the ACMG-23 panel⁴. Another recent study of over 13,000 screened couples with an ethnic distribution representative of the U.S. population found that 31% of at-risk couples would have been undetected using the ACMG-23, which would have resulted in missed cases of cystic fibrosis³.

Even more concerning is that most currently used targeted cystic fibrosis screening panels are at least five years old and were designed based on the CFTR2 database. Though these panels contain many more variants than the ACMG-23 panel, they still lack sufficient carrier screening coverage for the diverse U.S. population (Table 1). This is due to their design based on outdated and ethnically homogeneous data. The lack of coverage for specific ethnicities can further be seen for an on-market panel in Table 2.

Table 1. Three on-market *CFTR* panels with more variants detected than ACMG 23, but lacking sufficient cystic fibrosis carrier detection for the diverse U.S. population.

Assay	Variants	U.S. Population Covered*	Cystic Fibrosis Carriers Missed*
<i>CFTR</i> Panel #1	74	87%	13%
<i>CFTR</i> Panel #2	97	88%	12%
<i>CFTR</i> Panel #3	139	88%	12%

*Calculated based on pathogenic alleles in the U.S. demographic from Beauchamp KA, et al. *Genet Med.* 2019

Table 2. Manufacturer-reported *CFTR* mutation detection rates of a commonly used *CFTR* targeted panel⁵. Reported coverage percentages are based on data published in 2004 or earlier, meaning coverage could be poorer if compared with more recently published, diverse datasets.

Ashkenazi Jewish	Caucasian	Hispanic	African American	Asian American
96%	91%	84%	73%	55%

However, universal carrier screening is recommended for cystic fibrosis, which means clinicians need results that accurately represent the risk of passing on this disease for patients of all ancestries.

Large-scale population studies are adding rapidly to the information available about pathogenic variants associated with cystic fibrosis in people of diverse genetic backgrounds. Some of these studies deliberately gather data from diverse populations, such as that of the United States^{3,4}. The rush of new, publicly-available genomic data represents significant progress in identifying relevant variants across ancestries.


Still, this information must be incorporated into carrier screening tests to make a difference for prospective parents wondering if their children would be at risk of inheriting cystic fibrosis. Clinicians ordering carrier screening tests for cystic fibrosis should reach out to their clinical lab teams to ensure that such tests have been recently updated to reflect more diverse data sources – covering not only the most common variants found in the CFTR2 database but also diverse population-specific variants identified in large-scale sequencing studies.

Spinal Muscular Atrophy Carrier Screening

Until recently, a diagnosis of spinal muscular atrophy (SMA) was considered a death sentence. This debilitating neuromuscular disease is a leading genetic cause of death among infants. The introduction of game-changing therapies in the last few years, though, has brought new hope to families of affected individuals¹⁶.

Though these therapies have been revolutionary, they do not negate the need for carrier screening. In fact, SMA carrier screening is recommended by the American College of Obstetricians and Gynecologists (ACOG) and ACMG for all women who are pregnant or planning to become pregnant^{1,17}. SMA has a high carrier rate of approximately 1 in 50 individuals¹⁸.

In addition, SMA screening accuracy can vary depending on ancestry. Historically, *SMN1* copy number was thought to be the only information needed for SMA carrier status: if a person had two copies of *SMN1* it was assumed they were on different alleles and the person could not pass on the disease to a child. Now it is known that conventional tests that report only *SMN1* copy number may miss “silent carriers”, or people who carry two copies of the *SMN1* gene on a single allele and no copies on the other allele (Figure 2). This silent carrier genotype can occur in any ethnicity, but it is more common among people of African ancestry⁹, so standard SMA screening tests may be more likely to miss carriers in this population. In fact, if reporting *SMN1* copy number alone, “silent carrier” genotypes can lead to approximately 30% false negative results for African American patients⁹.



If measuring *SMN1* copy number alone, “silent carrier” genotypes can lead to up to **~30% false negative results**, depending on ethnicity¹⁰

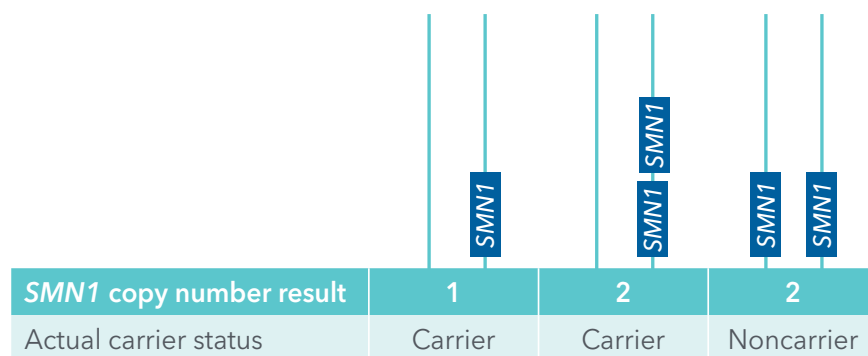


Figure 2. Illustration of *SMN1* “silent carrier” genotype with two *SMN1* copies on the same allele (center), which can allow for passing on a zero copy allele, compared to a noncarrier genotype where one *SMN1* copy is present on each allele (right). One copy *SMN1* carrier genotype is on left. If receiving *SMN1* copy number results only, silent carriers cannot be distinguished since both center and right genotypes would yield results of 2 copies of *SMN1*.

Fortunately, it has been observed that certain variants (called “linked variants”) in the *SMN1* gene can help predict silent carrier status. Presence of these variants can help further stratify carrier risk in all ethnicities, and even diagnose carrier status in a few^{19,20} (Table 3).

Table 3. Impact of positive results for an *SMN1* linked variant (c.*3+80T>G) on SMA carrier risk^{19,20} when positive for two *SMN1* copies, by ethnicity.

Ethnicity	Population Carrier Frequency	Residual Risk when 2 <i>SMN1</i> Copies Detected	Residual Risk after Positive for <i>SMN1</i> c.*3+80T>G
Ashkenazi Jewish	1 in 41.1	1 in 345	Carrier
Asian	1 in 53	1 in 628	Carrier
Spanish*	1 in 40	1 in 781	Carrier
African American	1 in 66	1 in 121	1 in 34
Hispanic	1 in 117	1 in 1061	1 in 140
Caucasian	1 in 35	1 in 632	1 in 29

*See reference 20; note that a limited number of 2/0 carriers with this variant were analyzed.

It is no longer enough to report only *SMN1* copy number for carrier screening. All SMA carrier screening tests should also include detection of these linked variants to provide patients with better information on potential carrier status.

Furthermore, many commonly used tests for SMA are not as accurate or as sensitive as they should be when providing such vital clinical information. The test considered the gold standard for SMA testing is based on a cumbersome, time-consuming technology called MLPA. There has been some concern cited that this technology may not reliably distinguish certain copy numbers²¹. In addition, popular SMA screening technologies may not provide linked variant detection to inform carrier risk. Clinicians should check with their clinical lab teams to ensure they are using an SMA carrier screening test capable of providing this information.

Fragile X Carrier Screening

Fragile X syndrome is the most common inherited form of intellectual disability and autism, affecting approximately 1 in 4,000 males and 1 in 5,000 females in the United States²². ACOG and ACMG recommend carrier screening for this syndrome^{1,17,23}. However, of the estimated 1 million women believed to be Fragile X carriers in the U.S.²², the majority may be unaware of their carrier status.

Fragile X syndrome is caused by a CGG repeat expansion in the *FMR1* gene on the X chromosome. Different counts of the repeat have different phenotypic outcomes (Table 4). Healthy individuals typically have fewer than 45 CGG repeats, while those who may be unaffected carriers or have associated disorders such as Fragile X-Associated Primary Ovarian Insufficiency (FXPOI) generally have between 55 and 200 repeats. Individuals with at least 200 repeats are diagnosed with Fragile X syndrome.

Table 4. Clinical categories and carrier status of mutations in the *FMR1* gene^{24,25,26}.

<i>FMR1</i> CGG Repeat Number	Clinical Category	Clinical Features	Carrier Status and Stability of <i>FMR1</i> CGG-Repeat region
5-44	Normal	None	Not a carrier of FXS. Stable allele. Allele passed from parent to child with rare changes in repeat size.
45-54	Intermediate	None	Not a carrier. Stable allele. Alleles occasionally undergo a small change in repeat size in some families.
55-199	Premutation	FXTAS symptoms over age 50 FXPOI in women	Carrier of FXS. Unstable allele with risk of large changes in repeat size from one generation to the next which can result in a full mutation and FXS. Risk of expansion varies based on total number of CGG repeats and AGG interruptions.
≥200	Full Mutation	Fragile X syndrome with or without autism	Unstable allele.

Abbreviations: FXS, fragile X syndrome; FXTAS, fragile X-associated tremor/ataxia syndrome; FXPOI, fragile X-associated primary ovarian insufficiency.

For any genetic testing platform, obtaining an accurate count of so many repeated DNA triplets is a serious challenge. Additionally, many analysis platforms cannot accurately interrogate the region due to the technical challenges of assessing GC-rich DNA content. Therefore, it is important that fragile X carrier screening is being performed with robust technology that can analyze extreme GC content and provide accurate counts of CGG repeats in order to identify women at risk of having a child with fragile X.

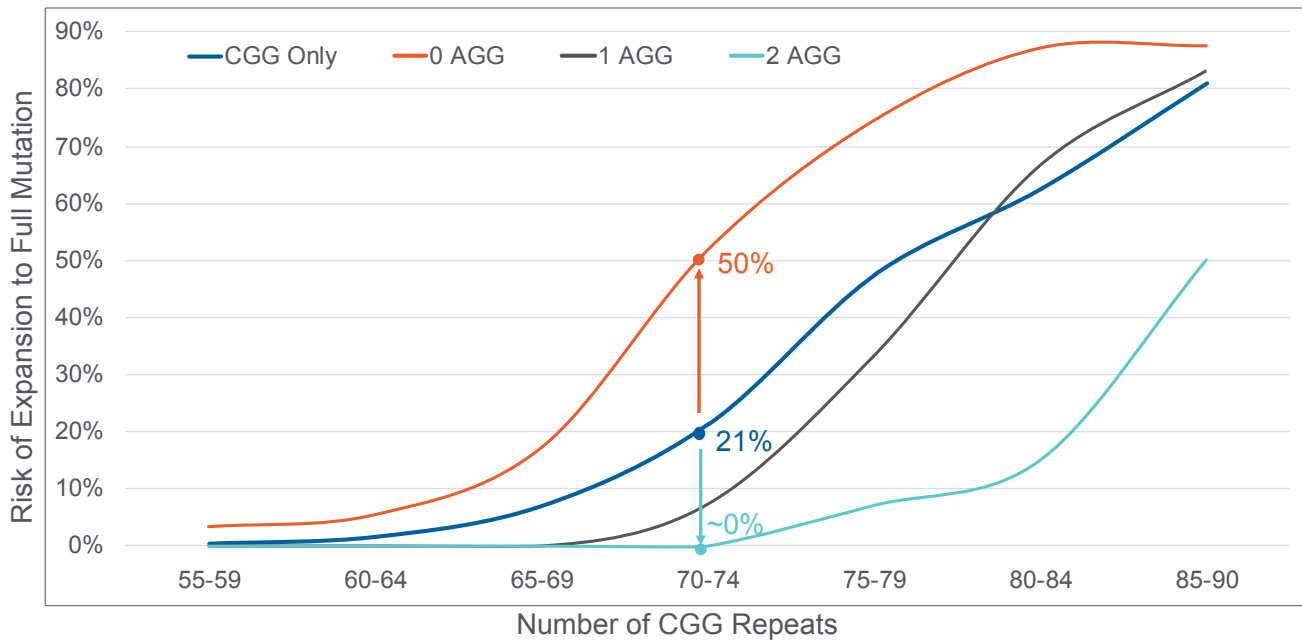


Figure 3. AGG interruption quantification stratifies risk of expansion to full mutation in the next generation^{10,11,27}. Risk by CGG repeat number only represented by blue line; 0 AGG interruptions detected, orange line; 1 AGG interruption detected, black line; 2 AGG interruptions detected, teal line. A 70 CGG patient example is illustrated where 21% risk is reported with CGG repeat-only information, which can be further stratified to range from 50% risk when 0 AGG interruptions (orange arrow) are detected to near 0% risk when 2 AGG interruptions are detected (teal arrow).

Beyond CGG repeat counts, clinicians should also be provided information on AGG interruptions for women with 50 - 90 CGG repeats. In this select group, the number of AGG interruptions stratifies risk of expansion to a full mutation in offspring beyond that provided by CGG repeats alone (Figure 3). For example, for a woman with a 70 CGG repeat allele, risk of expansion to a full mutation can range from ~0% to 50% depending on the number of AGG interruptions. Information provided by the AGG interruption status can greatly affect reproductive decisions; thus, clinicians should work with their clinical lab partners to ensure they are providing this information for select patients.

Conclusion

As insight into inherited diseases grows and diagnostic technology advances, providing all patients with accurate and relevant information must remain the central goal. Thankfully, due to the diligence of many scientists and clinicians, better tests have been developed for cystic fibrosis, spinal muscular atrophy, and fragile X carrier screening. As guidelines have shifted to pan-ethnic carrier screening, it is vital that clinicians and their clinical lab partners are aware of disparities and gaps in genetic information from often-used, yet outdated tests, and that they strive to provide more comprehensive and reliable information for all patients.

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