CF Mutation Coverage

A Year's Experience with Next Generation Full Gene Sequencing

NxGen MDx

Introduction

Cystic fibrosis (CF) is a fatal hereditary disease in the U.S. CF is a disorder of the cells that line the lungs, small intestines, sweat glands, and pancreas. The current average life expectancy for someone with CF is 37 years. Cystic fibrosis is caused by variants in the CF transmembrane regulator (CFTR) gene, located on the long arm of chromosome 7. An individual who inherits two pathogenic variants in the CFTR gene will have CF or a CFTR-related disorder. Twelve million Americans, or 1 in 25, are symptomless carriers of pathogenic variants in the CFTR gene, making it the most common genetic disorder in the U.S.

Cystic fibrosis, the most common genetic disorder in the U.S., is caused by pathogenic variants in the CFTR gene. 1 in 25 people are symptomless carriers of a variant in this gene.

According to a revised Committee Opinion issued by The American College of Obstetricians and Gynecologists (ACOG), prenatal and preconception CF carrier screening should be made available to all women of reproductive age as a routine part of obstetric care.1 However, prenatal and pre-conception CF carrier screening have been routine in obstetric practice as early as 2001 with the goal of identifying couples at risk of having a child with classic CF. CF affects people of different ethnicities with varying frequency, although it is increasingly difficult to assign individuals to a single ethnicity. It is reasonable, therefore, to offer CF carrier screening to all patients regardless of ethnicity.1 The sensitivity of genotyping-based screening tests, however, varies greatly among different ethnic groups. Sensitivities could range from less than 50% in those of Asian ancestry to 95% in Caucasians. This wide gap in sensitivity means that in some individuals, up to half of all variants that cause cystic fibrosis in their ethnicity are not assessed.

NxGen MDx opened its doors in 2013 with the idea of bringing ethnicity-agnostic carrier screening to the market. After looking closely at what was currently being offered in the carrier screening market and the limitations that were present, it was clear that sequencing every exon and splice-overlap intron region of each gene would offer the most comprehensive carrier screening. The methodology best suited to this full gene sequencing approach was next-generation sequencing (NGS). The first carrier screening test developed at NxGen with the full-gene sequencing approach was the NxGen MDx Cystic Fibrosis Carrier Screen. The NxGen CF Carrier Screen utilizes NGS technology to sequence all the exons, splice sites, and promoter regions of the CFTR gene for the most comprehensive carrier screen available.

Data

There are currently over 270 known pathogenic variants in the CFTR gene that can cause loss of function of the CFTR protein severe enough to cause the hallmark buildup of mucus in the lung associated with cystic fibrosis. Currently, ACOG recommends testing for 23 of the most common CF-causing variants.
The detection rate and residual risk across 5 different ethnic backgrounds for a 23-variant test and the NxGen CF Carrier Screen is illustrated in Table 1. Residual risk disparity is most dramatic in Hispanic White, African American, and Asian ethnicities.

There are 270 known variants for cystic fibrosis. ACOG recommends testing for 23 of the most common CF-causing variants, but testing for only these few variants leaves a huge gap in sensitivity among ethnicities. There is a dramatic residual risk disparity most notably between Hispanic White, African American, and Asian ethnicities.

Very few U.S. labs offer just the ACOG 23-variant panel any more. Recognizing the growing genetic diversity of the population, they have replaced it with expanded genotyping assays that test for more variants. Though these assays do result in better residual risks following negative test results, the only way to cover all genetic heritages without bias is to sequence the full CFTR gene. After 3 years of CF carrier screening using this methodology, we examined the list of variants detected over that time period to see how many would have been found be the commonly available genotyping panels (Table 2).

Table 1: Carrier Frequency Detection Rates and Residual Risk By Ethnicity

Ethnic Background	Carrier Risk Before Screening	23-Variant Detection Rate (%)	Residual Risk After Negative Test Result	NxGen MDx Cystic Fibrosis Carrier Screen Detection Rate (%)	Residual Risk After Negative Test Result
Ashkenazi Jewish	1/24	94	1/380	99	1/2301
Non-Hispanic White	1/25	88	1/200	99	1/2401
Hispanic White	1/58	72	1/200	99	1/5701
African American	1/61	64	1/170	99	1/6001
Asian American	1/94	49	1/180	99	1/9301

Discussion

4 in 10 people identify as multiracial in the United States. As the population becomes more diverse, providing residual risk calculations and knowing which genes to test is challenging. But because full gene sequencing is ethnicity agnostic, it has equal sensitivity within all genetic heritages. This also makes the test "future proof," meaning that as new variants are found, full-gene sequencing will already cover them.

For a given carrier screen, concern over disparities in residual risk between ethnicities is perhaps self-explanatory. What does bear further discussion is how these residual risk disparities impact individuals with diverse genetic heritages. The multiracial birth rate has grown from just 1% in 1970 to 9% in 2000. 2 Of these individuals, only 4 in 10 identify as multiracial. 3 This presents a challenge for physicians, genetic counselors, and laboratories trying to provide patients with accurate residual risk calculations following carrier screening. As the genetic diversity of the U.S. population continues to grow, this issue will become more and more challenging.

This is further illustrated by looking at the data in Table 2. Even a panel including over 600 CFTR variants did not cover all variants. Surprisingly, there was even one variant covered in the 159 variant panel that was not covered in the 600 variant panel. This serves to illustrate the difficulty of determining which variants to cover in a limited genotyping panel. Acording to NCBI's ClinVar database, the CFTR gene contains over 2000 identified variants, some pathogenic and some not. A systematic and in-depth review of these variants would be needed when determining which to include in a genotyping panel and even with that review, new clinical studies often change the pathogenic determination for variants necessitating development of a new panel.

Full gene sequencing offers the opportunity to leave these weaknesses of targeted genotyping behind. Sequencing all the areas of a gene where pathogenic variants can occur allows a test to have equal sensitivity within all genetic heritages. This simplifies the residual risk calculation by removing the variable of uneven sensitivity across different ethnicities. It also allows a genetic carrier screen to be more "future proof." With ever increasing data available on the human genome and disease-causing variants, keeping diagnostic laboratory assays current with the available knowledge can be challenging and costly. By design, full gene sequencing addresses all likely regions where pathogenic variants could occur in a gene so as new variants are found, the assay will already cover them.

Table 2: Pathogenic Variants By CF Screen Size

Mutation	Protein	Clinical Significance	NxGen CF Carrier Screen	34 Mutations	97 Mutations	141 Mutations	159 Mutations	600 Mutations
c.254G>A	p.Gly85Glu	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.273+3A>C	No Protein Name	Pathogenic	Yes	No	Yes	No	No	No
c.349C>T	p.Arg117Cys	Pathogenic	Yes	No	Yes	Yes	Yes	Yes
c.617T>G	p.Leu206Trp	Pathogenic	Yes	No	Yes	Yes	Yes	Yes
c.650A>G	p.Glu217Gly	Pathogenic	Yes	No	No	No	No	No
c.100T>T	p.Ile336Lys	Pathogenic	Yes	No	No	Yes	Yes	Yes
c.1040G>A	p.Arg347His	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1040G>C	p.Arg347Pro	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1520_1522delTCT	dF508	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1558G>T	p.Val520Phe	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1585-1G>A	No Protein Name	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1624G>T	p.Gly542Ter	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1646G>A	p.Ser549Asn	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1652G>A	p.Gly551Asp	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1657C>T	p.Arg553Ter	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.1675G>A	p.Ala559Thr	Pathogenic	Yes	No	Yes	Yes	Yes	Yes
c.1684G>A	p.Val562Ile	Pathogenic	Yes	No	No	No	No	No
c.1853T>C	p.Ile618Thr	Pathogenic	Yes	No	No	No	No	No
c.1865G>A	p.Gly622Asp	Pathogenic	Yes	No	No	No	No	No
c.2052_2053insA	p.Gln685ThrfsX4	Pathogenic	Yes	No	No	Yes	Yes	Yes
c.2249C>T	p.Pro750Leu	Pathogenic	Yes	No	No	No	No	No
c.2490+1G>A	No Protein Name	Pathogenic	Yes	No	No	Yes	Yes	Yes
c.2657+2_2657+3insA	No Protein Name	Pathogenic	Yes	No	No	No	Yes	No
c.2657+5G>A	No Protein Name	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.2735C>A	p.Ser912Ter	Pathogenic	Yes	No	No	No	No	Yes
c.2988+1G>A	No Protein Name	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.3140-26A>G	No Protein Name	Pathogenic	Yes	No	No	Yes	Yes	Yes
c.3197G>A	p.Arg1066His	Pathogenic	Yes	No	No	Yes	Yes	Yes
c.3205G>A	p.Gly1069Arg	Pathogenic	Yes	N No	No	Yes	Yes	No
c.3528delC	p.Lys1177Serfs	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.3846G>A	p.Trp1282Ter	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes
c.3884_3885insT	p.Ser1297PhefsX 5	Pathogenic	Yes	No	No	Yes	Yes	Yes
c.3909C>G	p.Asn1303Lys	Pathogenic	Yes	Yes	Yes	Yes	Yes	Yes

References:

1. The American College of Obstetricians and Gynecologists Committee Opinion Number 486 April 2011. Update on Carrier Screening for Cystic Fibrosis.
2. Pew Research Center tabulations of the 1979, 1980, 1990, and 2000 censuses and 2010 and 2013 American Community Surveys (IPUMS).
3. Pew Research Center survey, Feb. 6 - April 6 2015 (n=1555 multiracial adults).
4. Modified from the American College of Medical Genetics. Technical Standards and Guidelines for CFTR Mutation Testing, 2006 Edition.