WOMEN'S HEALTH

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One Lab's Experience with Cystic Fibrosis Carrier Screening in Males with Infertility

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Introduction:

Cystic fibrosis (CF) is caused by variants in the CFTR gene with over 1,700 reported variants. It is known that males with CF have a higher risk of infertility.

In 2004, the American College of Medical Genetics and Genomics (ACMG) recommended that a minimum panel of 23 CFTR disease-causing variants be utilized for population carrier screening with reflex poly T testing if the R117H variant is identified.[1] Since then, laboratory panels with a minimum of 23 variants have become widely available.

In the context of male infertility, there is limited consensus across professional societies regarding both test methodology and the minimum number of CFTR variants that should be examined for these patients.

- The American Society for Reproductive Medicine (ASRM) recommends males with certain indications (congenital absence of the vas deferens (CBAVD), azoospermia secondary to congenital bilateral obstruction of the epididymides and unilateral vasa agenesis) undergo CF testing, however does not specify the number of CFTR variants nor the methodology (genotyping versus sequencing).[2]
- The American Urology Association (AUA) best practice statement for evaluation of infertile males specifies that males with CBAVD should be offered CF genetic testing with poly T analysis but also does not specify a minimum number of variants or test methodology.[3]
- The American College of Obstetrics and Gynecology (ACOG) committee opinion #691 recommends males with CBAVD be referred for genetic consultation with consideration of CFTR sequencing.[4]

Given the wide range of guidelines, we aim to describe our laboratory's experience with CFTR genotyping in males with infertility and the proportion of CFTR variants that would not have been identified using the 23 variant panel.

Methods:

We queried a population of patients who underwent either the CF 40 variant panel or the Expanded CF panel (200+ variants) between October 2012 and August 2019 at GenPath.

A subset of male patients were categorized as having infertility based on the provided clinical indication(s) or specialty of the ordering provider. Clinical indications were obtained using provided ICD10 codes such as E29.1 (male infertility, unspecified), N46.01 (organic azoospermia), and many other infertility-associated codes. The specialty of the ordering provider was elucidated using key terms such as "infertility" and "IVF" in the practice name to identify those who are likely fertility specialists. For example, if the name of the ordering provider's practice was "Infertility Specialists R Us", we categorized their male patients as possibly having infertility. While this approach was likely appropriate, it is possible that some male patients were miscategorized as having infertility when they do not since, for example, patients might have seen a fertility-related specialist for routine carrier screening.

From this population, we identified males who tested positive for two CFTR variants. We reviewed these variants to determine if they were included in the ACMG recommended 23 variant panel (Table 1). Of note, since reflex poly T testing is recommended if the R117H variant is identified, we counted the 5T variant as part of the ACMG panel for the purposes of our study.

Table 1 ACMG Recommended 23 CFTR Variants

c.1585-1G>A	p.Ala455Glu (A455E)	p.Gly542Ter (G542X)	c.1766+1G>A
p.Arg1162Ter (R1162X)	p.Gly551Asp (G551D)	c.2657+5G>A	p.Arg117His (R117H)
p.Gly85Glu (G85E)	c.2988+1G>A	p.Arg334Trp (R334W)	p.Ile507del (ΔI507)
c.3717+12191C>T	p.Arg347Pro (R347P)	p.Lys1177fs (c.3659delC)	c.489+1G>T
p.Arg553Ter (R553X)	p.Lys684fs (c.2184delA)	c.579+1G>T	p.Arg560Thr (R560T)
p.Phe508del (ΔF508)	IVS9>-5T (5T)*	p.Asn1303Lys (N1303K)	p.Trp1282Ter (W1282X)

1. List of 23 CFTR variants recommended for testing by the 2004 ACMG Guidelines
2. *5T variant counted as part of the ACMG recommended panel for this study
3. ■: Variants identified in the 10 males with infertility indication and 2 *CFTR* variants

Results:

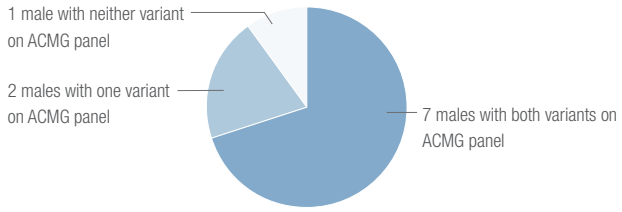
A total of 17,109 males had CFTR genotyping of whom 5,160 (30.2%) were identified as infertility patients. Within this subpopulation of males with infertility, 10/5,160 (0.2%) tested positive for 2 CFTR variants (either homozygous or compound heterozygous) (Figure 1).

Figure 1 Positive CF Yield in Men with Infertility Indication

17,109	All males having CF targeted *CFTR* variant testing
5,160 (30.2%)	Of males tested, proportion with infertility indication
10 (0.2%)	Of males tested for infertility indication, number with two *CFTR* variants

If these 10 males with 2 CFTR variants had undergone testing for only the 23 variant panel, 7/10 (70%) would have been identified as carrying 2 variants, 2/10 (20%) would have been identified as carrying only 1 variant and possibly presumed to be a carrier, and 1/10 (10%) would have had a negative result (Figure 2).

Figure 2 Ten Males with Infertility Indication and Two *CFTR* Variants



Of note, 2/7 males with 2 variants were positive for the R117H and 5T variants, with phase unknown. On a variant level, 10 unique *CFTR* variants were identified in this subpopulation of homozygous/compound heterozygous males with infertility, of which 4 (40%) are not included in the 23 variant panel. These four variants were:

- c.3067_3072del (p.Ile1023_Val1024del)
- c.2353C>T (p.Arg785X)
- c.2875delG (p.Ala959fs)
- c.1721C>A (p.Pro574His)

Conclusion:

For males with infertility, expanding CF genetic testing beyond the minimum 23 variants would increase the identification of males with clinically significant CFTR variants, however there is limited consensus regarding CF genetic testing guidelines to assist providers who manage these patients.

Existing CF panels were designed for reproductive carrier screening and as demonstrated by Claustres et al. males with infertility are more likely to have 2 CFTR variants that may not be detectable by these panels.[5]

In our review of 10 homozygous or compound heterozygous males with infertility, 3 patients would have received incomplete results by testing for only 23 variants. Additionally, poly T analysis is recommended by ACMG as a reflex test if the R117H variant is identified. While this approach is appropriate for carrier screening, males with the 5T variant in combination with a second CFTR variant can have a range of phenotypes that include infertility and non-classic CF. Thus, for males with infertility who undergo the 23 variant panel and test negative for the R117H variant, poly T analysis would not be performed and clinically significant CFTR variant(s) may not be identified.

Creation of more specific CF genetic testing guidelines for males with infertility would aid providers in managing this population of patients.

References:

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