Novel pathogenic variants are routinely detected even in extensively-sequenced genes, such as *CFTR*

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Background

Cystic fibrosis (CF) is caused by pathogenic variants in the *CFTR* gene and is one of the most common autosomal recessive genetic disorders. Carrier screening for CF is recommended for all couples considering a pregnancy, regardless of ethnicity. However, many carrier screening laboratories only offer testing for limited panels of the most prevalent pathogenic variants, resulting in CF screening tests with lower detection rates in non-Caucasian populations.

Objective

To report our experience identifying novel pathogenic variants in *CFTR* during routine carrier screening.

Materials & Methods

Using a clinical NGS-based assay, we sequenced the coding region of *CFTR*, intron-exon boundaries, and select intronic variants in a large pan-ethnic population of individuals referred for carrier screening. As shown in Figure 1, sequencing results were filtered for known pathogenic variants, as well as previously unreported ("novel") variants predicted to be pathogenic based on their truncating effect (nonsense, frameshift, and canonical splice site variants). All reported variants were confirmed by Sanger sequencing.

Figure 1. Variant Filtration for Carrier Screening

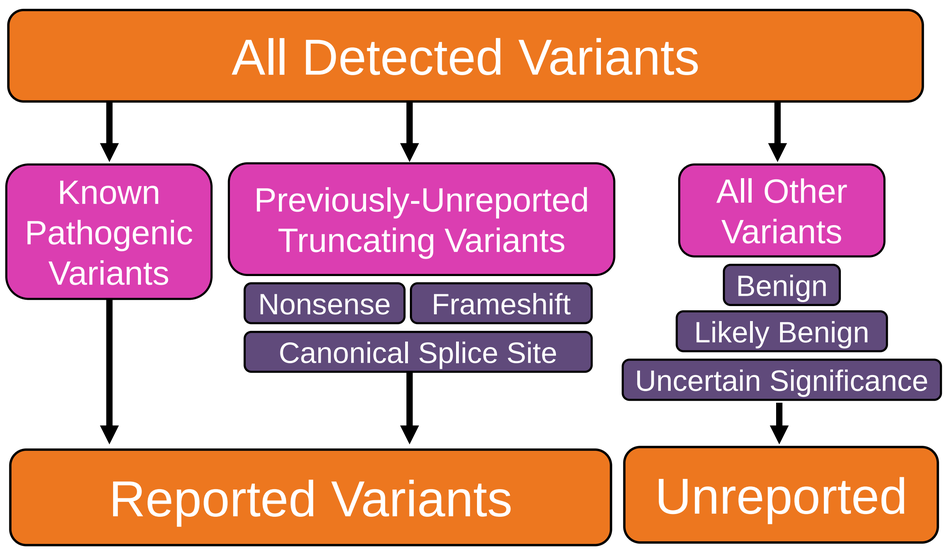
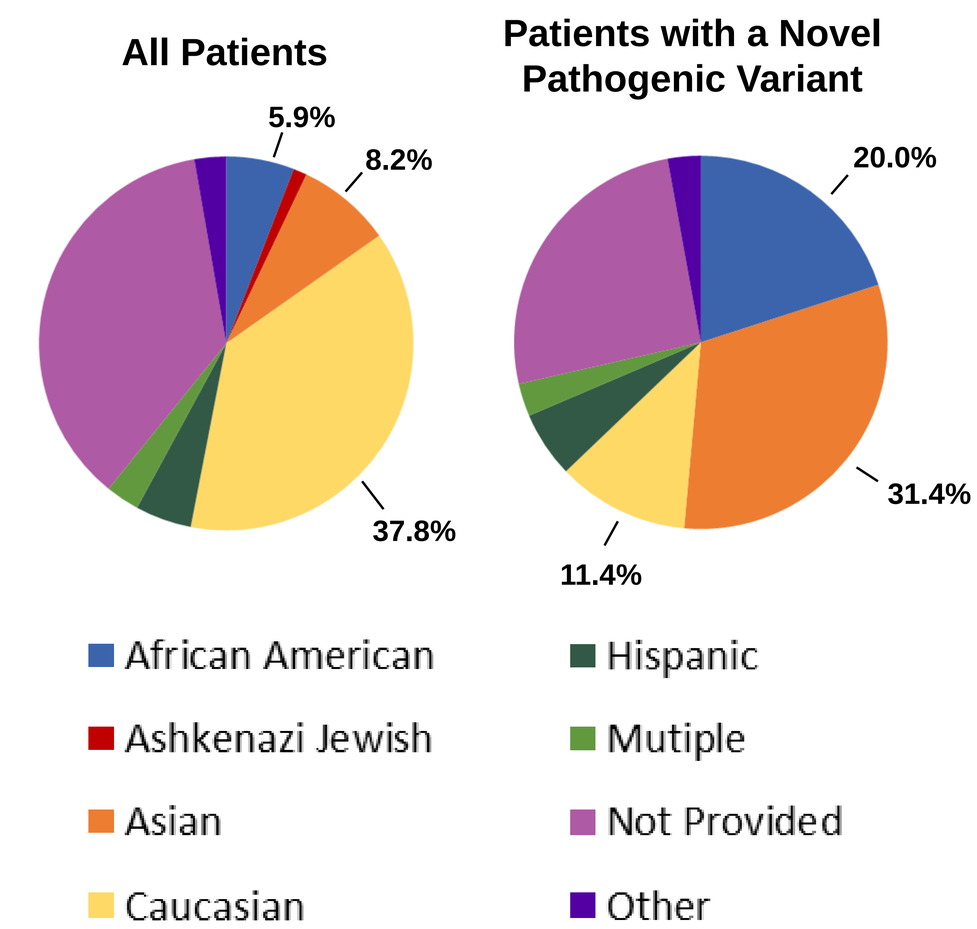


Figure 2. Reported Ethnicities of Tested Patients



Results

Despite the fact that *CFTR* has been extensively studied with >2000 variants identified to date, we detected 34 different novel truncating *CFTR* variants in our patients (Table 1). One novel variant was found in two individuals; all others were detected only once. Self-reported ethnicities of the 35 carriers included 11 Asian, 7 African American or African, 4 Caucasian, 2 Hispanic, 1 South East Asian, 9 individuals who did not provide their ethnicity, and 1 person who selected multiple ethnicities. As shown in Figure 2, this ethnicity distribution differed significantly from that of our overall patient population.

Of the 34 unique variants detected, 8 were nonsense variants, 24 were frameshifts, and 2 were located in canonical splice sites. Of note, we did not detect any mutation hot spots, as novel variants were distributed throughout the gene. Four variants were detected in the last exon of the gene, but all are located before the last truncating variant reported in a patient.

Table 1. *CFTR* Variants Reported as Novel Pathogenic

Patient	Novel Variant Detected (NM_000492.3)	Exon/ Intron	Reported Patient Ethnicity	Reproductive Partner Results (if available)**
1	c.51delC*	1	African American	
2	c.580G>T (p.Gly194X)	6	Hispanic	
3	c.874_875delGA*	8	African American	
4	c.1012_1013delAC	8	Multi-Ethnic	(Egg Donor)
5	c.1130delA	9	Caucasian	Negative (TS)
6	c.1210-2A>G	Intron 9	Caucasian	
7	c.1253delA	10	Not Provided	Negative (TS)
8	c.1293dupT	10	Asian	
9	c.1420G>T (p.Glu474X)	11	Asian	Negative (TC)
10	c.1526delG	11	Asian	Negative (TS)
11	c.1579G>T (p.Glu527X)	11	Not Provided	
12	c.1807delG*	14	African American	
13	c.1943delA*	14	Asian	Negative (TS)
14	c.2089delA	14	African American	Negative (TS)
15	c.2333delA	14	Not Provided	
16	c.2475_2478dupCGAA	14	Not Provided	
17	c.2745_2746delGT	17	Not Provided	Negative (TC)
18	c.2901_2908+6del	17	Asian	CARRIER (TS) c.1210-2A>C
19	c.2901_2908+6del	17	Asian	
20	c.2909-1G>C	Intron 17	Not Provided	Negative (TS)
21	c.3078delT	19	Caucasian	Negative (TS)
22	c.3110C>A (p.Ser1037X)	19	Caucasian	Negative (TC)
23	c.3454_3455delGA	21	Asian	Negative (TF)
24	c.3563C>A (p.Ser1188X)	22	Asian	(Sperm Donor)
25	c.3639dupA	22	Not Provided	
26	c.3743C>G (p.Ser1248X)*	23	African American	Negative (TS)
27	c.3827C>G (p.Ser1276X)	23	South East Asian	Negative (TS)
28	c.3851_3852dupAA	23	Not Provided	
29	c.4028dupG	25	Asian	Negative (TC)
30	c.4036dupC	25	Hispanic	
31	c.4204delC	26	Not Provided	
32	c.4252delG	27	African	
33	c.4297G>T (p.Glu1433X)	27	African American	Negative (TC)
34	c.4350delT	27	Asian	CARRIER (TF) c.349C>T (p.Arg117Cys)
35	c.4374dupC	27	Asian	

* These variants were subsequently reported in CF patients [Schrijver (2016) JMD 18(1):39-50], further affirming their pathogenicity.
** **TC** = Partners Tested Concurrently; **TF** = Partner Tested First; **TS** = Partner Tested Second (after reporting of novel variant result)

Clinical Notes

Patient 17

F508del was also detected in this patient. He was reportedly diagnosed with CF at a young age and followed by a specialist, but had not had previous molecular testing.

Patient 29

Patient has CBAVD. CFTR c.4056G>C (p.Gln1352His) detected in trans. PolyT testing was negative (7T/7T).

Patient 34

Couple later had a son with negative IRT on CA NBS, but genetic testing had not yet been done on the child.

Conclusions

It is remarkable that, in a well-studied gene like *CFTR*, we identified 35 carriers of pathogenic variants that had not been previously reported in the literature. Even the most comprehensive targeted panel of known pathogenic variants would have missed these carriers. Novel truncating variants were preferentially detected in non-Caucasian individuals, highlighting both the need for better characterization of the variant spectrum in these populations and the importance of offering them a truly pan-ethnic carrier screening test. Additionally, screening for large (exon-level) deletions and duplications across the *CFTR* gene can further enhance detection rates by identifying carriers of both rare and novel changes. We recently added del/dup analysis to our CF carrier screening test and have identified at least three carriers of multi-exon deletions.

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