Frequency of Cystic Fibrosis Transmembrane Conductance Regulator Variants in Individuals Evaluated for Primary Ciliary Dyskinesia

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Objective To evaluate whether cystic fibrosis transmembrane conductance regulator (*CFTR*) variants are more common among individuals tested for primary ciliary dyskinesia (PCD) compared with controls.

Study design Data were studied from 1021 individuals with commercial genetic testing for suspected PCD and 91 777 controls with genetic testing at the same company (Invitae) for symptoms/diseases unrelated to PCD or *CFTR* testing. The prevalence of *CFTR* variants was compared between controls and each of 3 groups of individuals tested for PCD (PCD-positive, -uncertain, and -negative molecular diagnosis).

Results The prevalence of 1 pathogenic *CFTR* variant was similar among the individual groups. When combining the PCD-uncertain and PCR-negative molecular diagnosis groups, there was a higher prevalence of single pathogenic *CFTR* variants compared with controls ($P = .03$). Importantly, >1% of individuals who had negative genetic testing results for PCD had 2 pathogenic *CFTR* variants (8 of 723), and the incidence of cystic fibrosis (CF) (2 pathogenic variants) is roughly 1 in 3000 individuals of Caucasian ethnicity (~0.03%). This incidence was also greater than that of 2 pathogenic *CFTR* variants in the control population (0.09% [84 of 91 777]; $P = 9.60 \times 10^{-16}$). These variants correlate with mild CFTR-related disease.

Conclusions Our results suggest that a single pathogenic *CFTR* variant is not likely to be a PCD-mimetic, but ongoing studies are needed in individuals in whom PCD is suspected and genetic testing results are uncertain or negative. Furthermore, CF may be misdiagnosed as PCD, reflecting phenotypic overlap. Among individuals evaluated for PCD, CF should be considered in the differential even in the CF newborn screening era. *(J Pediatr 2019;215:172-7)*.

Primary ciliary dyskinesia (PCD; MIM 244400) is characterized by a defect in motile cilia[1,2] and is associated with otosinopulmonary disease,[1-3] heterotaxy,[4] neonatal respiratory distress,[5] and diminished fertility.[6,7] There is phenotypic overlap between PCD and cystic fibrosis (CF; MIM 219700) (**Table I**).[8] Patients with CF have impaired mucociliary clearance associated with defective anion permeability across epithelial membranes.[9] Some individuals present with atypical CF,[10] and a cystic fibrosis transmembrane conductance regulator (*CFTR*)-related metabolic syndrome (CRMS) can occur in individuals with intermediate sweat chloride values.[11] Atypical CF and CRMS can be missed on newborn screening.

Electron microscopy findings are often falsely positive or negative in patients evaluated for PCD, and recent guidelines recommend that a PCD diagnosis requires at least 2 classic clinical PCD findings, coupled with an abnormal nasal nitric oxide and/or abnormal electron microscopy findings, and/or a diagnostic genotype.[4,12] Thus, most PCD centers request genetic testing early during the evaluation of a patient considered likely to have PCD. However, genetic defects have been identified in only ~65% of patients with PCD,[1] and the correct diagnosis can remain elusive for many patients. An increased CF carrier frequency has been reported among individuals with asthma,[13] and genes encoding ciliary proteins have been identified as modifiers of CF lung disease.[14] Therefore, we hypothesized that *CFTR* haploinsufficiency could be observed in patients with clinical suspicion of PCD but with nondiagnostic PCD genetic testing. Next-generation sequencing allows for interrogation of *CFTR* variants in samples sent for PCD testing. Surprisingly, we found that ~1% of subjects evaluated for PCD actually had 2 pathogenic *CFTR* variants.

CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CRMS	Cystic fibrosis transmembrane conductance regulator–related metabolic syndrome
PCD	Primary ciliary dyskinesia
polyT	Polythymidine
TG	Thymidine and guanine
VUS	Variant of uncertain significance

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Methods

Invitae (San Fransisco, California) performed genetic testing in evaluation of PCD (**Table II**; available at www.jpeds.com) for 1021 individuals from 2014 to 2017.[15] Invitae uses next-generation sequencing, and analysis of *CFTR* was possible. According to an Institutional Review Board–approved protocol, we performed deidentified retrospective analysis of the *CFTR* variants in all individuals tested for PCD at Invitae. There were also 91 777 control *CFTR* genotypes available from patients who underwent genetic testing for diseases/symptoms unrelated to PCD or *CFTR* during the same period. The majority of these controls had undergone testing for cancer syndromes, cardiac conditions, and neurologic conditions. Individuals with genetic testing for pancreatitis or gastrointestinal cancer were excluded from the control population, because these conditions are more common in patients with CF than in the general population. Any individual with a test requisition for genetic testing of *CFTR* was excluded from this control dataset.

Variant classification was performed as described previously.[16] In brief, each variant was classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign. Classification was based on confidence that a variant was likely to cause disease as recommended by joint consensus guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[17] In this study, "pathogenic" variants refer to variants that have been classified as pathogenic or likely pathogenic at Invitae.

Individuals who had undergone genetic testing to evaluate possible PCD were stratified into 3 cohorts: PCD-positive, PCD-uncertain, and PCD-negative (**Table III**). Not all individuals with PCD genetic testing were tested for the same number of genes because of improvements in the sequencing assay design. For the 32 PCD genes studied, the number of individuals with sequencing data for each gene is shown in **Table II**. Individuals were also stratified based on *CFTR* variants as 2 pathogenic, 1 pathogenic, uncertain, or negative (**Table II**).

Some *CFTR* variants are associated with milder disease and/or incomplete penetrance. Specifically, deleterious variants that have been shown to retain ~10%-25% of CFTR activity relative to wild-type are often associated with variable expressivity and penetrance.[18,19] Attention was given to the classification of the polythymidine variants. Variations in 2 regions of intron 9 of *CFTR*, known as the polyT (polythymidine) and TG (thymidine and guanine) tracts, have a complex association with disease. Most individuals have 1 of 3 different polyT lengths on each copy of the gene: T5, T7, or T9. The T5 variant can affect *CFTR* splicing efficiency and is associated with the full spectrum of disease, from severe CF to asymptomatic.[19-23] The T7 and T9 variants are considered benign; they do not lead to clinical symptoms. The TG tract is upstream of the polyT tract. The length of the TG tract modifies the severity and penetrance of the T5 variant[19,22-26]; in most individuals, its length ranges from 10 to 13 TG repeats. When a T5 is next to a TG13 (TG13T5) or TG12 (TG12T5), the phenotype is associated with CF or *CFTR*-related disorders > 80% of the time.[22,25,26] When the T5 is next to a TG11 (TG11T5), the effect on *CFTR* splicing efficiency is less common, and this is associated with milder *CFTR*-related disorders with lower penetrance (<50%). T5 next to a TG10 (TG10T5) does not lead to clinical symptoms. Thus, the TG13T5 and TG12T5 variants are classified as pathogenic at Invitae, and the TG11T5 variant is classified as pathogenic (low penetrance). Because the TG11T5 variant can be asymptomatic, individuals with this variant are included here in the "uncertain" findings group.

When the R117H *CFTR* variant is on the same allele as ("in *cis* with") TG12T5, together these variants disrupt CFTR

Table I. Phenotypic differences and similarities between PCD and CF

PCD	Clinical features common to both conditions	CF
Laterality defects	Recurrent ear infections	Pancreatic insufficiency
Neonatal respiratory distress	Recurrent pneumonia	Liver disease
Congenital heart disease	Bronchiectasis	Diabetes
	Sinus infections	Meconium ileus
	Infertility	Distal intestinal obstruction

Table III. Description of cohorts of PCD genetic testing results and *CFTR* genetic testing results

Category	Definition
PCD-positive	Two pathogenic variants in the same gene or a homozygous variant; males with 1 pathogenic variant in an X-linked PCD gene
PCD-uncertain	Two VUSs in the same gene; 1 pathogenic variant, with or without a concurrent VUS in the same gene; males with a single VUS in an X-linked PCD gene
PCD-negative	A single VUS in 1 or more autosomal recessive gene; only likely benign and/or benign variants
CFTR 1 pathogenic	A single *CFTR* pathogenic variant, including TG13T5 and TG12T5
CFTR uncertain	Uncertain significance and/or low-penetrance *CFTR* variants, including TG11T5 and R117H
CFTR 2 pathogenic	Two pathogenic *CFTR* variants including TG13T5 and TG12T5 or a homozygous pathogenic *CFTR* variant

more than the TG12T5 variant alone[27-30]; this may result in increased severity of *CFTR*-related symptoms.[31,32] The impact of having R117H in *cis* with a T7 or T9 is currently unclear, however.[30-32] As a result, Invitae has classified this as a VUS. We were unable to determine the phase of TG12T5 and R117H variants in this study: those with only R117H were included in the uncertain group; those with TG12T5 and R117H were included in the 1 pathogenic group; and those with the TG12T5 variant, 1 additional pathogenic variant, and the R117H variant composed the 2 pathogenic group. In individuals who are homozygous for a specific TGT5 variant and heterozygous for a R117H variant we can assert that the TGT5 and R117H variants are in *cis*. There were 3 such individuals (2 controls and 1 PCD-negative), each of whom was homozygous for TG12T5 and heterozygous for R117H. These 3 individuals are included in the 2 pathogenic *CFTR* variant cohort, given the variant classifications used. No individual was both homozygous for TG13T5 or TG11T5 and also heterozygous for an R117H variant.

A trend test was used to compare the frequency of 1 pathogenic *CFTR* variant among the patients with PCD-positive, -uncertain, and -negative molecular diagnoses. The Pearson χ^2 test was performed to compare the frequency of *CFTR* variants between the patients who were PCD-positive and controls, between the patients who were PCD-uncertain and controls, and between the PCD-negative patients who were and controls. Finally, the Pearson χ^2 test was used to compare the prevalence of 1 pathogenic *CFTR* variant among all individuals with nondiagnostic genetic testing for PCD (combining the PCD-unknown and -negative molecular diagnosis cohorts) and controls. For all statistical analyses comparing the frequency of 1 pathogenic *CFTR* variant, individuals with 2 pathogenic *CFTR* variants were excluded.

Results

Comparing the frequency of individuals with a single pathogenic *CFTR* variant among the PCD-positive, -uncertain, and -negative molecular diagnosis cohorts revealed no significant differences (Table III). There was also no difference in the frequency of carriers with a single pathogenic *CFTR* variant when comparing the PCD-positive, -uncertain, or -negative molecular diagnosis group with the control population (Table IV). However, when the PCD-uncertain and -negative molecular diagnosis cohorts were combined into a single group, there was a small but statistically significant enrichment of carriers with a single pathogenic *CFTR* variant compared with controls ($P = .03$, Pearson χ^2 test).

In our analysis, it was apparent that >1% of individuals in the PCD-negative molecular diagnosis cohort had 2 pathogenic *CFTR* variants (8 of 723 individuals, compared with 84 of 91 777 controls; $P = 9.60 \times 10^{-16}$). This may be an underestimate, because individuals with both a pathogenic variant and a TG11T5 variant were excluded from the 2 pathogenic *CFTR* variants cohort (Table V; available at www.jpeds.com).

The average age of the PCD negative molecular testing cohort was 14.5 years, and the median age was 6 years (Table VI; available at www.jpeds.com). The average age of controls was significantly greater than that of the individuals tested for PCD, likely because of the later age of diagnostic testing for the control cohort, which consisted mainly of individuals evaluated for cancer, heart disease, and neurologic disorders. The CFTR2 database demonstrates that some of the 8 *CFTR* genotypes of individuals with negative PCD genetic testing and 2 pathogenic *CFTR* variants are associated with borderline sweat chloride values and sometimes with preserved pancreatic function (Table VII). This database, when first published in 2013, provided phenotypic data for 39 696 individuals with CF, stratified by *CFTR* genotype.[27] Interestingly, 4 of the 8 individuals had at least 1 3849 + 10kbC > T variant (1 individual was homozygous for this variant), which can be associated with atypical CF (Table VII). Some of the genotypes are not sufficiently common to have determinate phenotypic data. As may be expected, lung function of individuals with these genotypes varies widely. Given that some of these *CFTR* genotypes are uncommon and others may be associated with borderline

Table IV. Frequency of *CFTR* genotypes stratified by PCD genetic test results

CFTR findings	PCD-positive molecular diagnosis	PCD-uncertain molecular diagnosis	PCD-negative molecular diagnosis	PCD-combined uncertain and negative molecular diagnosis	Controls
1 pathogenic variant, n (%)	11 (6.5)	10 (7.8)	53 (7.3)	63 (7.4)	5272 (5.8)
Uncertain, n (%)	17 (9.9)	17 (13.4)	69 (9.5)	86 (10.1)	10 123 (11.0)
2 pathogenic variants, n (%)	0	1 (0.8)	8 (1.1)	9 (1.1)	84 (0.1)
Negative, n (%)	143 (83.6)	99 (78.0)	593 (82.0)	692 (81.4)	76 298 (83.1)
Total, n	171	127	723	850	91 777
P value, 1 pathogenic variant compared with controls	.70	.29	0.57	**.03**	
P value, 2 pathogenic variants compared with controls	1*	.26*	**9.60×10^{-16}**	**$<2.2 \times 10^{-16}$**	

P values are provided for each group based on PCD genetic test results (positive, uncertain, or negative) comparing the frequency of 1 pathogenic *CFTR* variant with controls and the frequency of 2 pathogenic *CFTR* variants with controls. Also, the frequency of *CFTR* variants among all individuals with nondiagnostic genetic testing (combining both PCD-uncertain and -negative molecular diagnosis cohorts into 1 group) was compared with controls. Significant *P* values are in bold type.
*Continuity-corrected *P* value.

Table VII. *CFTR* genotype of 8 individuals with 2 pathogenic *CFTR* variants and negative PCD molecular testing

	First *CFTR* variant	Second *CFTR* variant	# individuals in CFTR2 with genotype	% with genotype in CFTR2 who are pancreatic insufficient	% with genotype in CFTR2 with *Pseudomonas* infection	Avg sweat chloride value (mEq/L) of individuals with genotype in CFTR2	FEV1% predicted for individuals < age 10 with genotype in CFTR2	FEV1 % predicted for individuals age 10-20 with genotype in CFTR2	FEV1 % predicted for individuals > age 20 with genotype in CFTR2
1	c. 3181G > C (p. Gly1061Arg)	c.3718-2477 C > T (legacy 3849 + 10kbC > T)	1	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data
2	c. 1521_1523delCTT (p. Phe508del) (legacy F508del)	c.3454G > C (p.Asp1152His)	358	27	36	44	81-117	70-122	35-108
3	c. 3718-2477C > T (legacy 3849 + 10 kb C > T)	c.3718-2477C > T (legacy 3849 + 10kbC > T)	56	30	45	53	Insufficient data	60-111	28-76
4	c. 1521_1523delCTT (p. Phe508del) (legacy F508del)	c.579 + 3 A > G (legacy 711 + 3 A > G)	42	20	40	65	102-119	Insufficient data	34-99
5	c. 3266 G > A (p. Trp1089X)	c.3718-2477 C > T (legacy 3849 + 10kbC > T)	4	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data
6	c. 1521_1523delCTT (p. Phe508del) (legacy F508del)	c.200C > T (p.Pro67Leu)	186	33	45	61	87-117	74-115	30-104
7	c. 3718-2477C > T (legacy 3849 + 10kbC > T)	c.1680-886A > G (legacy 1811 + 1.6 kbA > G)	2	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data
8*	TG12T5	TG12T5	3†	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data

CFTR2 was queried to obtain genotype–phenotype correlation data. For each of the 8 *CFTR* genotypes, data from CFTR2 is provided regarding pancreatic insufficiency, % with *Pseudomonas* infection, average sweat chloride values, and FEV_1 % predicted data.
*There was also an individual with this *CFTR* genotype who had uncertain PCD molecular testing.
†Although additional individuals in CFTR2 have a 5T genotype without a known TG tract genotype, only 3 individuals are known to be TG12T5 homozygous.

sweat chloride values or pancreatic sufficiency, it would not be surprising if some of these individuals evaluated for PCD may have undiagnosed *CFTR*-related disease that could be missed by newborn screening protocols and with symptoms that may present at a later age than classic CF.

Discussion

The role of a single pathogenic *CFTR* variant in causing PCD-like symptoms. No individual PCD group (positive, uncertain, or negative molecular diagnosis) had a significant enrichment of individuals with a single pathogenic *CFTR* variant compared with controls. However, when combining the PCD-uncertain and PCD-negative groups (individuals with nondiagnostic genetic testing), there was a small but statistically significant ($P = .03$) enrichment for 1 pathogenic *CFTR* variant compared with controls, suggesting that pathogenic variants in *CFTR* may contribute to the clinical presentation or severity of PCD-like symptoms. Ongoing studies of patients with PCD-like symptoms are needed to address the possibility that *CFTR* haploinsufficiency could contribute to PCD-like symptoms.

Frequency of *CFTR* Variants in a Symptomatic Population with PCD

A previous study suggested that prevalent *CFTR* variants in the Polish population were not seen more commonly in individuals with PCD.[33] We had access to genetic testing results from much larger suspected PCD and control cohorts, and we conducted this analysis with all observed pathogenic *CFTR* variants. Consistent with the previous study, we did not find an enrichment of heterozygous pathogenic *CFTR* variants in the PCD- positive molecular diagnosis cohort.

Frequency of CF in a Population of Patients with Suspected PCD

More than 1% of individuals with negative PCD genetic testing were found to have 2 pathogenic *CFTR* variants and a likely diagnosis of *CFTR*-related disease. The prevalence of 2 pathogenic *CFTR* variants is also greater than that observed in the general population, ~1 in 3000 individuals of Caucasian ethnicity. This could be an underestimate, given that we are using a conservative classification of pathogenic variants. For instance, 2 individuals in the PCD-negative molecular diagnosis cohort had 1 clearly pathogenic *CFTR* variant and the reduced penetrance TG11T5 variant but were not included in the 2 pathogenic *CFTR* variant group, given how we classify the different T5 variants (**Table V**). Some *CFTR* VUS may also be pathogenic, but evidence is conflicting and/or insufficient at this time. Therefore, at a minimum, we expect 1% of individuals with negative PCD genetic testing to have a positive molecular diagnosis of *CFTR*-related disease. This likely reflects the phenotypic overlap of these 2 conditions (otosinopulmonary disease and infertility; **Table I**). Thus, the possibility of an atypical or mild form of CF missed on newborn screen should always be considered in patients with PCD symptoms, particularly in the absence of heterotaxy/laterality defects. *CFTR* genetic testing can be included with PCD genetic testing.

Limitations

This retrospective study used deidentified data, and thus we were unable to obtain specific clinical information for individuals with 2 pathogenic *CFTR* variants. As discussed above, it is likely that some individuals with negative PCD genetic testing and 2 pathogenic *CFTR* variants have atypical CF (as is expected to be true of a portion of individuals within the control population with 2 pathogenic *CFTR* variants), but we cannot know this for certain. Furthermore, the phase of the pathogenic *CFTR* variants remains unknown, meaning that we cannot be sure the variants are biallelic. Finally, it remains possible that ethnic differences between the PCD and control groups affected the prevalence of individuals with a single pathogenic *CFTR* variant among these groups.

Future studies evaluating phenotype and frequency of *CFTR* variants in individuals with positive PCD molecular test results may be particularly interesting, considering that this study was not capable of testing the hypothesis that a pathogenic *CFTR* variant may be a modifier of PCD phenotypic severity. With the advent of a PCD patient registry through the PCD Foundation, such an experiment may soon be feasible. ■

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References

1. Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia: recent advances in diagnostics, genetics, and characterization of clinical disease. Am J Respir Crit Care Med 2013;188:913-22.
2. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, et al. Primary ciliary dyskinesia: diagnostic and phenotypic features. Am J Respir Crit Care Med 2004;169:459-67.
3. Sommer JU, Schäfer K, Omran H, Olbrich H, Wallmeier J, Blum A, et al. ENT manifestations in patients with primary ciliary dyskinesia: prevalence and significance of otorhinolaryngologic co-morbidities. Eur Arch Otorhinolaryngol 2011;268:383-8.
4. Shapiro AJ, Zariwala MA, Ferkol T, Davis SD, Sagel SD, Dell SD, et al. Diagnosis, monitoring, and treatment of primary ciliary dyskinesia: PCD Foundation consensus recommendations based on state of the art review. Pediatr Pulmonol 2016;51:115-32.
5. Mullowney T, Manson D, Kim R, Stephens D, Shah V, Dell S. Primary ciliary dyskinesia and neonatal respiratory distress. Pediatrics 2014;134:1160-6.
6. Munro NC, Currie DC, Lindsay KS, Ryder TA, Rutman A, Dewar A, et al. Fertility in men with primary ciliary dyskinesia presenting with respiratory infection. Thorax 1994;49:684-7.
7. Raidt J, Werner C, Menchen T, Dougherty GW, Olbrich H, Loges NT, et al. Ciliary function and motor protein composition of human fallopian tubes. Hum Reprod 2015;30:2871-80.
8. Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet 2015;16:45-56.
9. Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. J Clin Invest 2002;109:571-7.

10. Paranjape SM, Zeitlin PL. Atypical cystic fibrosis and CFTR-related disease. Clin Rev Allergy Immunol 2008;35:116-23.
11. Farrell PM, White TB, Derichs N, Castellani C, Rosenstein BJ. Cystic fibrosis diagnostic challenges over 4 decades: historical perspectives and lessons learned. J Pediatr 2017;181S:S16-26.
12. Shapiro AJ, Davis SD, Polineni D, Manion M, Rosenfeld M, Dell SD, et al. Diagnosis of primary ciliary dyskinesia. An official American Thoracic Society clinical practice guideline. Am J Respir Crit Care Med 2018;197:e24-39.
13. Nielsen AO, Qayum S, Bouchelouche PN, Laursen LC, Dahl R, Dahl M. Risk of asthma in heterozygous carriers for cystic fibrosis: a meta-analysis. J Cyst Fibros 2016;15:563-7.
14. Blue E, Louie TL, Chong JX, Hebbring SJ, Barnes KC, Rafaels NM, et al. Variation in cilia protein genes and progression of lung disease in cystic fibrosis. Ann Am Thorac Soc 2018;15:440-8.
15. Truty R, Paul J, Kennemer M, Lincoln SE, Olivares E, Nussbaum RL, et al. Prevalence and properties of intragenic copy-number variation in Mendelian disease genes. Genet Med 2019;21:114-23.
16. Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Ho YY, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genet Med 2017;19:1105-17.
17. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
18. Raraigh KS, Han ST, Davis E, Evans TA, Pellicore MJ, McCaque AF, et al. Functional assays are essential for interpretation of missense variants associated with variable expressivity. Am J Hum Genet 2018;102:1062-77.
19. Niksic M, Romano M, Buratti E, Pagani F, Baralle FE. Functional analysis of cis-acting elements regulating the alternative splicing of human CFTR exon 9. Hum Mol Genet 1999;8:2339-49.
20. Delaney SJ, Rich DP, Thomson SA, Hargrave MR, Lovelock PK, Welsh MJ, et al. Cystic fibrosis transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels. Nat Genet 1993;4:426-31.
21. Strong TV, Wilkinson DJ, Mansoura MK, Devor DC, Henze K, Yang Y, et al. Expression of an abundant alternatively spliced form of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is not associated with a cAMP-activated chloride conductance. Hum Mol Genet 1993;2:225-30.
22. Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. Am J Hum Genet 2004;74:176-9.
23. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, et al. Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros 2011;10(suppl 2):S86-102.
24. Cuppens H, Lin W, Jaspers M, Costes B, Teng H, Vankeerberghen A, et al. Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. J Clin Invest 1998;101:487-96.
25. Sun W, Anderson B, Redman J, Milunsky A, Buller A, McGinniss MJ, et al. CFTR 5T variant has a low penetrance in females that is partially attributable to its haplotype. Genet Med 2006;8:339-45.
26. Salinas DB, Azen C, Young S, Keens TG, Kharrazi M, Parad RB. Phenotypes of California CF newborn screen-positive children with CFTR 5T allele by TG repeat length. Genet Test Mol Biomarkers 2016;20:496-503.
27. Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet 2013;45:1160-7.
28. Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild-disease-form Cl- channels with altered pore properties. Nature 1993;362:160-4.
29. Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO3- transport in mutations associated with cystic fibrosis. Nature 2001;410:94-7.
30. de Nooijer RA, Nobel JM, Arets HG, Bot AG, van Berkhout FT, de Rijke YB, et al. Assessment of CFTR function in homozygous R117H-7T subjects. J Cyst Fibros 2011;10:326-32.
31. Thauvin-Robinet C, Munck A, Huet F, Génin E, Bellis G, Gautier E, et al. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. J Med Genet 2009;46:752-8.
32. Waller MD, Simmonds NJ. Phenotypic variability of R117H-CFTR expression within monozygotic twins. Paediatr Respir Rev 2016;20(suppl):21-3.
33. Skrzypczak U, Rutkiewicz E, Pogorzelski A, Witt M, Zietkiewicz E. Carrier status for 3 most frequent CFTR mutations in Polish PCD/KS patients: lack of association with the primary ciliary dyskinesia phenotype. J Appl Genet 2007;48:85-8.

Table II. The 32 genes included in PCD genetic testing

Gene	Individuals with PCD- positive molecular testing	Individuals with PCD- uncertain molecular testing	Individuals with PCD- negative molecular testing
ARMC4	170	126	718
CCDC103	170	126	718
CCDC114	170	126	718
CCDC151	170	126	718
CCDC39	171	127	723
CCDC40	171	127	723
CCDC65	170	126	718
CCNO	170	126	718
DNAAF1	171	127	723
DNAAF2	171	127	723
DNAAF3	170	126	718
DNAAF5	170	126	718
DNAH1	91	63	300
DNAH11	171	127	723
DNAH5	171	127	723
DNAH8	170	126	718
DNAI1	171	127	723
DNAI2	171	127	723
DNAL1	171	127	723
DRC1	170	126	718
GAS8	91	63	300
LRRC6	91	63	300
MCIDAS	170	126	718
NME8	170	126	718
OFD1	171	127	723
RPGR	170	126	718
RSPH1	170	126	718
RSPH3	91	63	300
RSPH4A	171	127	723
RSPH9	171	127	723
SPAG1	170	126	718
ZMYND10	170	126	718

Not all individuals with PCD genetic testing were tested for the same number of PCD genes due to revisions and improvements in the sequencing assay design. For each of 32 PCD genes studied, the number of individuals with sequencing data is shown.

Table V. Number of individuals with T5 variant(s) (stratified by TG tract) and R117H variant(s) in the PCD-positive, PCD-uncertain, and PCD-negative molecular testing groups and controls

Variants	PCD-positive (n = 171)	PCD-uncertain (n = 127)	PCD-negative (n = 723)	Controls (n = 91 777)	Classification
2 TG13T5	0	0	0	2	2 pathogenic variants
2 TG12T5	0	1	1	22	2 pathogenic variants
2 TG11T5	0	0	0	94	Uncertain
2 R117H	0	0	0	0	Uncertain
TG13T5 + TG12T5	0	0	0	3	2 pathogenic variants
TG13T5 + TG11T5	0	0	0	2	1 pathogenic variant
TG12T5 + TG11T5	0	0	0	49	1 pathogenic variant
TG13T5 + R117H	0	0	0	0	1 pathogenic variant
TG12T5 + R117H	1	0	1	23	1 pathogenic variant
TG11T5 + R117H	0	0	0	13	Uncertain
TG13T5 + other pathogenic *CFTR* variant	0	0	0	2	2 pathogenic variants
TG12T5 + other pathogenic *CFTR* variant	0	0	0	39	2 pathogenic variants
TG11T5 + pathogenic *CFTR* variant	0	0	2	107	1 pathogenic variant
R117H + pathogenic *CFTR* variant	0	0	0	3	1 pathogenic variant
1 TG13T5	0	0	4	187	1 pathogenic variant
1 TG12T5	2	5	20	2014	1 pathogenic variant
1TG11T5	9	13	34	5090	Uncertain
1 R117H	0	0	0	309	Uncertain

The number of individuals is provided for each genotype (for individuals with 2 different *CFTR* variants, phase is not known). The *CFTR* classification used in this study is also provided for each of these genotypes (2 pathogenic variants, 1 pathogenic variant, or uncertain).

Table VI. Demographic data for each cohort

Cohorts	Median age, y	Mean age, y	Self-reported white ethnicity, %
PCD-positive molecular testing	16	20.4	53.8
PCD-uncertain molecular testing	9	16.9	54.3
PCD-negative molecular testing	6	14.5	59.5
Control population	51	48.8	61.2