CFTR Genotype and Maximal Exercise Capacity in Cystic Fibrosis

A Cross-Sectional Study

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Abstract

Rationale: Cystic fibrosis transmembrane conductance regulator (CFTR) is expressed in human skeletal muscle cells. Variations of CFTR dysfunction among patients with cystic fibrosis may be an important determinant of maximal exercise capacity in cystic fibrosis. Previous studies on the relationship between *CFTR* genotype and maximal exercise capacity are scarce and contradictory.

Objectives: This study was designed to explore factors influencing maximal exercise capacity, expressed as peak oxygen uptake ($\dot{V}O_{2peak}$), with a specific focus on *CFTR* genotype in children and adults with cystic fibrosis.

Methods: In an international, multicenter, cross-sectional study, we collected data on *CFTR* genotype and cardiopulmonary exercise tests in patients with cystic fibrosis who were ages 8 years and older. *CFTR* mutations were classified into functional classes I–V.

Results: The final analysis included 726 patients (45% females; age range, 8–61 yr; forced expiratory volume in 1 s, 16 to 123% predicted) from 17 cystic fibrosis centers in North America, Europe, Australia, and Asia, all of whom had both valid maximal cardiopulmonary exercise tests and complete *CFTR* genotype data. Overall, patients exhibited exercise intolerance ($\dot{V}O_{2peak}$, 77.3 ± 19.1% predicted), but values were comparable among different *CFTR* classes. We did not detect an association between *CFTR* genotype functional classes I–III and either $\dot{V}O_{2peak}$ (percent predicted) (adjusted $\beta = -0.95$; 95% CI, −4.18 to 2.29; $P = 0.57$) or maximum work rate ($Watt_{max}$) (adjusted $\beta = -1.38$; 95% CI, −5.04 to 2.27; $P = 0.46$) compared with classes IV–V. Those with at least one copy of a *F508del-CFTR* mutation and one copy of a class V mutation had a significantly lower $\dot{V}O_{2peak}$ ($\beta = -8.24\%$; 95% CI, −14.53 to −2.99; $P = 0.003$) and lower $Watt_{max}$ (adjusted $\beta = -7.59\%$; 95% CI, −14.21 to −0.95; $P = 0.025$) than those with two copies of a class II mutation. On the basis of linear regression analysis adjusted for relevant confounders, lung function and body mass index were associated with $\dot{V}O_{2peak}$.

Conclusions: *CFTR* functional genotype class was not associated with maximal exercise capacity in patients with cystic fibrosis overall, but those with at least one copy of a *F508del-CFTR* mutation and a single class V mutation had lower maximal exercise capacity.

Keywords: cystic fibrosis transmembrane conductance regulator; peak oxygen uptake; lung disease; cardiorespiratory fitness

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Members of the CFTR-Exercise Study Group can be found before the References.

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Cystic fibrosis (CF) is a disorder with autosomal recessive inheritance that ultimately leads to respiratory failure and premature death. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene coding for the CFTR protein that functions as an ion channel, mainly for chloride and bicarbonate (1). *CFTR* mutations are grouped into different classes based on their effect on CFTR protein production, trafficking, function, and stability (1). CFTR is expressed in cell membranes of epithelial cells. It can also be found at the level of the sarcoplasmic reticulum in airway smooth muscle (2), human skeletal muscle cells (3, 4), and myocardium (5), but the precise physiological role of CFTR in these tissues is not fully understood (6). Previous studies suggest intrinsic functional abnormalities in skeletal muscles lacking functional CFTR (3, 4), possibly based on a disturbed calcium homeostasis in the muscle and increased systemic inflammation (3). It is therefore plausible that dysfunctional CFTR in human skeletal muscle could be a factor contributing to peripheral muscle weakness (7, 8) and reduced exercise capacity (e.g., peak oxygen uptake [$\dot{V}O_{2peak}$]) in CF (8, 9).

Because $\dot{V}O_{2peak}$ is a predictor of mortality in CF (10), knowledge of important correlates of aerobic performance may help to guide patient care. Few, predominantly small, studies have previously been done to investigate the relationships between *CFTR* genotype and $\dot{V}O_{2peak}$ in patients with CF (11–14), with equivocal and controversial results. Selvadurai and colleagues (12) found that children with CF carrying an *F508del-CFTR* gene mutation on one allele and a class I or class II *CFTR* mutation on the second allele had lower $\dot{V}O_{2peak}$ (approximately 30 to 45%) and peak anaerobic exercise capacity (approximately 10 to 17%) than children with a class III, IV, or V mutation on the second *CFTR* allele. However, this finding was based on univariate analysis of a relatively small sample size and thus did not control for relevant determinants of exercise capacity, such as nutritional status and chronic infection with *Pseudomonas aeruginosa* (8, 13, 15), which could have influenced their study findings and interpretation.

The present study was designed to investigate factors associated with $\dot{V}O_{2peak}$ (primary endpoint) and maximum work rate ($Watt_{max}$; secondary endpoint) with a specific focus on *CFTR* genotype in a large international cohort of children and adults with CF. We aimed to compare maximal exercise capacity among patients with *CFTR* mutations that result in severely reduced function (combinations of classes I–III mutations) with that of patients with combinations of classes IV–V mutations characterized by some residual CFTR function at the cell surface (16).

Methods

Study Design and Patients

We invited members from the Exercise Working Group of the European Cystic Fibrosis Society to participate in the study. We also searched the PubMed database for publications on exercise testing in CF to identify clinical studies that included cardiopulmonary exercise testing (CPET). The CPET had to be performed on a cycle ergometer employing the Godfrey cycle protocol (17) or a modification thereof. Inclusion and exclusion criteria and detailed information on CPET can be found in the online supplement.

We collected data on anthropometric characteristics, CF-related comorbidities (e.g., exocrine pancreatic insufficiency, cystic fibrosis–related diabetes [CFRD], colonization with *P. aeruginosa*), pulmonary function, CPET-related data, and genetic data. Chronic *P. aeruginosa* infection was considered to be present when more than 50% of at least four sputum samples collected in the previous year were positive (18). Spirometry was performed according to American Thoracic Society/European Respiratory Society standards (19). We calculated percent predicted values for spirometry (20), $\dot{V}O_{2peak}$ (21, 22), $Watt_{max}$ (21, 23), and peak heart rate (24), as well as *z*-scores for body mass index (BMI) based on World Health Organization criteria (25). Percent body fat was calculated using sex- and age-specific prediction equations (26), and lean body mass was derived from body fat and weight. For the final models, $\dot{V}O_{2peak}$ (primary endpoint) and $Watt_{max}$ (secondary endpoint) were expressed as percent predicted of reference values (22, 23).

The classification of *CFTR* genotype (27, 28) was performed by a geneticist (S.G.) who was blinded to the exercise testing data. Details about the functional classification of *CFTR* alleles are shown in Table E1 in the online supplement. Study participants were grouped for the analysis of the primary endpoint ($\dot{V}O_{2peak}$ percent predicted) in patients with both *CFTR* alleles in either class I, II, or III (corresponding to severely reduced CFTR function) and patients with at least one mutant allele in class IV or class V (corresponding to some residual CFTR function) (16). In an exploratory analysis, we also compared maximal exercise capacity between patients with at least one copy of the *F508del-CFTR* mutation and categorized them into five groups on the basis of their second *CFTR* mutation class (11, 27).

Ethical approval for this study was obtained from the cantonal ethical committee of Zurich, Switzerland (2015-0109). All centers obtained ethical approval (if required) for the use of their anonymized patient data according to national and local policies.

Statistical Analysis

Details on the statistical analyses can be found in the online supplement. We used analysis of variance, Kruskal–Wallis tests, and χ^2 tests to compare variables between groups. We used mixed-effects models with a random intercept for study center to identify characteristics associated with $\dot{V}O_{2peak}$ and $Watt_{max}$ (percent predicted). Multilevel mixed-effects models with a random intercept for each study center were used to examine associations between *CFTR* genotype-based group and

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$\dot{V}O_{2peak}$ and $Watt_{max}$ (percent predicted). The models were *a priori* adjusted for the variables age, sex, BMI *z*-score, and forced expiratory volume in 1 second (FEV_1; percent predicted) because it is generally accepted that they have an influence on $\dot{V}O_{2peak}$ (9, 29, 30). According to the results reported by van de Weert–van Leeuwen and colleagues (13), chronic infection with *P. aeruginosa* is associated with decline in $\dot{V}O_{2peak}$ over time and therefore represents an important covariate in our model. To decide if the potential confounders CFRD or pancreatic insufficiency should be taken into account, the Akaike information criterion was used for model choice. However, the models did not improve when we added those variables, for which reason they were discarded. We also checked if interactions between *CFTR* genotype-based group and the other influential variables improved the models, which was not the case. To confirm our approach of defining the exercise capacity outcome variables as percent predicted values, we additionally performed linear mixed models using a multiplicative, allometric approach to account for potential effects of body size and pulmonary function on the relationship between *CFTR* genotype and $\dot{V}O_{2peak}$ (*see* online supplement).

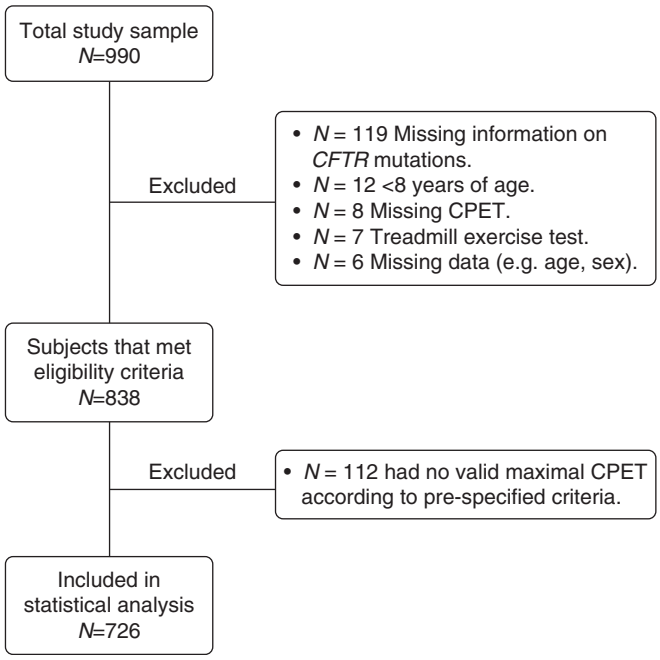

Figure 1. Flowchart of included patients. CFTR = cystic fibrosis transmembrane conductance regulator; CPET = cardiopulmonary exercise test.

Results

We contacted 32 centers in North America, Europe, Australia, and Asia by e-mail, 17 of which provided data for this study. Reasons for nonparticipation can be found in the online supplement. Included centers were in Canada ($n = 3$; 293 patients); the United States ($n = 2$; 110 patients); and one center each from Austria ($n = 30$ patients), Australia ($n = 30$ patients), France ($n = 59$ patients), Germany ($n = 69$ patients), Greece ($n = 39$ patients), Israel ($n = 37$ patients), Italy ($n = 34$ patients), the Netherlands ($n = 93$ patients), Spain ($n = 51$ patients), Serbia ($n = 64$ patients), Switzerland ($n = 42$ patients), and the United Kingdom ($n = 39$ patients). A flowchart of included patients is shown in Figure 1, and patient characteristics are shown in Table 1. Of 838 patients, 112 did not reach their maximal exercise level during the CPET and were excluded from further analyses. Criteria for a maximal effort during CPET are provided in the online supplement. These 112 patients were, on average, older, had lower FEV_1, lower exercise capacity, and more CF-related comorbidities (Table 1). The lower exercise capacity in patients not performing maximally was not explained by a pulmonary function limitation (i.e., a lower breathing reserve at end exercise [$\dot{V}E_{peak}$/predicted maximum voluntary ventilation] than the group demonstrating maximal effort) (Table 1). The final dataset included 726 patients (45% females; age, 18.7 ± 8.5 yr; age range, 8–61 yr; FEV_1, 76.6 ± 22.9; FEV_1 range, 16–123; $\dot{V}O_{2peak}$, 77.3 ± 19.1% predicted; $\dot{V}O_{2peak}$ range, 25–137). Figure 1 in the online supplement shows the relationship between FEV_1 and $\dot{V}O_{2peak}$. Table 2 shows data for maximal exercise capacity, lung function, nutritional status, and CPET-related variables grouped by *CFTR* mutation class.

Predictors of Maximal Exercise Capacity in Patients with Cystic Fibrosis

In the univariate analysis adjusted for study center, we did not find any association of *CFTR* functional class (I–III vs. IV–V) with either $\dot{V}O_{2peak}$ percent predicted ($\beta = 2.35$; 95% CI, −1.27 to 6.35; $P = 0.19$) or $Watt_{max}$ percent predicted ($\beta = 2.34$; 95% CI, −1.90 to 6.58; $P = 0.28$). In contrast, age, FEV_1 percent predicted, BMI *z*-score, CFRD, and chronic *P. aeruginosa* infection were associated with $\dot{V}O_{2peak}$ and $Watt_{max}$ (expressed as percent predicted). Exocrine pancreatic insufficiency was associated only with $Watt_{max}$ (percent predicted). Forced vital capacity was not included in the final multilevel multivariate model, owing to a high collinearity with FEV_1 ($R = 0.89$; $P = 0.001$). We noted differences in $\dot{V}O_{2peak}$ and $Watt_{max}$ among study centers. Consequently, center was included as a random intercept in the multilevel mixed-effects models. In fully adjusted models, FEV_1 percent predicted ($\beta = 0.41$; 95% CI, 0.36 to 0.47; $P < 0.001$) and BMI *z*-score ($\beta = 1.75$; 95% CI, 0.76 to 2.74; $P = 0.001$) were significantly associated with $\dot{V}O_{2peak}$ (percent predicted). In the model for $Watt_{max}$ (percent predicted), FEV_1 percent predicted ($\beta = 0.46$; 95% CI, 0.40–0.52; $P < 0.001$) and BMI *z*-score ($\beta = 1.27$; 95% CI, 0.15–2.38; $P = 0.03$) remained the only significant predictors.

Associations of CFTR Functional Class and Maximal Exercise Capacity

The prevalence of CFRD, pancreatic insufficiency, and *P. aeruginosa* infection were higher for patients with only classes I–III *CFTR* mutations than for patients

Table 1. Clinical characteristics and cardiopulmonary exercise testing data for patients with valid versus nonvalid maximal exercise tests

Variable	*n*	Valid Maximal CPET	*n*	Nonvalid Maximal CPET
Age, yr*	726	16.4 (13.0, 22.1)	112	20.9 (13.9, 28.8)
Sex, % females	726	330 (45)	112	45 (40)
Body mass index, *z*-score†	726	−0.24 (−0.92, 0.47)	112	−0.01 (−0.75, 0.70)
FEV_1, % predicted†	724	79 (66, 91)	111	74 (56, 86)
Cystic fibrosis–related diabetes, *n* (%)†	723	79 (11)	109	20 (18)
Pseudomonas aeruginosa, *n* (%)*	716	371 (52)	105	83 (79)
Pancreatic insufficiency, *n* (%)	724	611 (84)	112	90 (80)
$\dot{V}O_{2peak}$, % predicted* (22)	726	79 ± 19	112	67 ± 15
$\dot{V}O_{2peak}$, % predicted* (21, 22)	726	80 ± 20	112	70 ± 15
$Watt_{max}$, % predicted* (23)	724	85 ± 22	112	74 ± 18
$Watt_{max}$, % predicted* (21, 23)	724	91 ± 23	112	82 ± 19
HR_{max}, % predicted*	725	93 (88, 96)	112	87 (80, 92)
Respiratory exchange ratio*	639‡	1.16 (1.10, 1.23)	36‡	0.99 (0.96, 1.01)
$\dot{V}E_{peak}/MVV_{pred}$*	725	86 (70, 102)	111	76 (63, 87)

Definition of abbreviations: CPET = cardiopulmonary exercise test; FEV_1 = forced expiratory volume in 1 second; HR_{max} = maximum heart rate; $\dot{V}E_{peak}$ = peak minute ventilation; $\dot{V}O_{2peak}$ = peak oxygen consumption; $Watt_{max}$ = maximum work rate.
Data are mean ± SD, median (interquartile range), or number (percent). Percent predicted values for $\dot{V}O_{2peak}$ were calculated using equations from Orenstein (22) and Jones and colleagues (21). Data for $\dot{V}O_{2peak}$ are shown separately according to Orenstein (22) and a combination of Orenstein (22) and Jones and colleagues (21) for patients aged 16 years or younger and patients older than 16 years of age, respectively. $Watt_{max}$ percent predicted values were calculated using equations from Godfrey and colleagues (23) and Jones and colleagues (21). Data are shown separately according to Godfrey and colleagues (23) and a combination of Godfrey and colleagues (23) and Jones and colleagues (21) for patients aged 16 years or younger and patients older than 16 years of age, respectively. Statistical comparisons between groups were performed using an independent *t* test, a nonparametric Mann–Whitney *U* test, or the χ^2 test as appropriate.
*$P < 0.001$.
†$P < 0.05$.
‡Respiratory exchange ratio data from two study centers were not available.

with at least one *CFTR* allele associated with residual function (class IV or class V). Univariate analysis revealed no differences in maximal exercise capacity (absolute and percent predicted $\dot{V}O_{2peak}$ and $Watt_{max}$) between the two groups (Table 3). In mixed-effects models, we did not detect an association between *CFTR* genotype functional class and either $\dot{V}O_{2peak}$ (percent predicted) (β = −0.95; 95% CI, −4.18 to 2.29; $P = 0.57$) or $Watt_{max}$ (β = −1.38; 95% CI, −5.04 to 2.27; $P = 0.46$). In these models, both lung function and nutritional status were associated with $\dot{V}O_{2peak}$ and $Watt_{max}$ (percent predicted) independent of CFTR function (Table 4). These results were confirmed when allometric modeling was applied (Table E2) and when exercise capacity was expressed per kilogram of body weight or lean body mass (data not shown). Moreover, the results were similar when the 112 patients with nonmaximal CPETs were included in the analysis. In 835 and 831 patients, we found no association between *CFTR* genotype functional class and either $\dot{V}O_{2peak}$ (percent predicted) (β = −1.95; 95% CI, −5.28 to 1.39; $P = 0.25$) or $Watt_{max}$ (β = −2.42; 95% CI, −5.97 to 1.48; $P = 0.24$), respectively.

In a subanalysis restricted to patients with preserved lung function (FEV_1, ⩾80% predicted) and nutritional status (BMI *z*-score, ⩾50th percentile), we found no between-group differences in maximal exercise capacity in either univariate or adjusted analysis (Tables E3 and E4). Furthermore, a subanalysis on patients with moderate to severe lung function impairment (FEV_1, ⩽60% predicted) did not show between-group differences in either $\dot{V}O_{2peak}$ or $Watt_{max}$ (percent predicted) (Tables E5 and E6).

Exercise Capacity among Patients with at Least One Copy of the *F508del-CFTR* Mutation

In 653 patients who had at least one copy of the *F508del-CFTR* (class II) mutation grouped in five classes according to their second allele, no differences among *CFTR* genotype and either $\dot{V}O_{2peak}$ or $Watt_{max}$ were observed (absolute values or % predicted) (*see* Table E7). In mixed models, patients with one copy of a class V mutation had a significantly lower $\dot{V}O_{2peak}$ than patients with two copies of a class II mutation (β = −8.24%; 95% CI, −14.53 to −2.99; $P = 0.003$) (*see* Table E8). Furthermore, $Watt_{max}$ values were lower for the group with one copy of a class V mutation (β = −7.59%; 95% CI, −14.21 to −0.95; $P = 0.025$). In addition, the same association was found for $\dot{V}O_{2peak}$ and $Watt_{max}$ (31) using allometric models (Table E9).

Discussion

In this largest international multicenter study of exercise testing in patients with CF to date, we focused primarily on the relationship between *CFTR* genotype and predictors of maximal exercise capacity in patients with CF. We found that severity of *CFTR* genotype, using different CFTR categorizations, combinations, and analytic approaches, was not associated with maximal exercise capacity. In contrast, pulmonary function and nutritional status, expressed as BMI, were strongly associated with exercise capacity.

Our data do not support a relevant role of *CFTR* genotype on maximal exercise capacity in patients with CF. However, some theoretical considerations deserve further explanation to shed light on

Table 2. Lung function, nutritional status, and exercise capacity in 726 patients according to *CFTR* mutation (functional classes I–V), based on functional defect of the milder of the two mutations

	CFTR Class I/I	CFTR Class ⩽II/II	CFTR Class ⩽III/III	CFTR Class ⩽IV/IV	CFTR Class ⩽V/V
No. of patients	32	550	39	63	42
Age, yr*	14.6 (12.7, 18.3)	16.2 (12.9, 21.3)	16.7 (12.1, 25.0)	16.6 (12.1, 25.0)	19.0 (14.8, 26.5)
Sex, % female	14 (44)	244 (44)	16 (41)	34 (54)	21 (50)
Cystic fibrosis–related diabetes, *n* (%)*	7 (22)	64 (12)	4 (10)	2 (3)	1 (2)
Pancreatic insufficiency, *n* (%)†	35 (97)	529 (93)	34 (89)	15 (24)	10 (24)
Pseudomonas aeruginosa, *n* (%)*	32 (100)	519 (95)	21 (55)	23 (37)	15 (36)
Body mass index,† kg/m^2	18.8 (16.9, 20.1)	19.3 (17.3, 21.5)	20.4 (17.5, 24.2)	20.6 (18.8, 23.0)	22.3 (19.2, 25.0)
Body mass index, *z*-score*	−0.35 (−1.05, 0.39)	−0.28 (−0.97, 0.35)	0.26 (−0.53, 1.04)	−0.20 (−0.76, 0.63)	0.08 (−0.79, 1.14)
Lean body mass, kg*	38.2 (30.0, 46.4)	41.3 (32.9, 50.3)	43.5 (32.1, 55.2)	42.3 (36.7, 52.8)	47.0 (40.4, 61.1)
Body fat, %†	17.2 ± 4.7	18.2 ± 5.7	19.9 ± 5.5	21.4 ± 6.4	22.4 ± 6.5
FEV_1, % predicted	80 (45, 93)	79 (60, 94)	78 (50, 90)	86 (72, 96)	80 (62, 94)
$\dot{V}O_{2peak}$, L/min	1.6 (1.3, 1.8)	1.7 (1.4, 2.3)	1.8 (1.3, 2.2)	1.8 (1.5, 2.3)	1.7 (1.3, 2.4)
$\dot{V}O_{2peak}$, % predicted (22)	74 ± 17	79 ± 19	78 ± 24	83 ± 18	74 ± 19
$\dot{V}O_{2peak}$, % predicted (21, 22)	74 ± 17	80 ± 19	79 ± 23	85 ± 18	78 ± 23
$\dot{V}O_{2peak}$, <82% predicted, *n* (%)	21 (66)	313 (57)	23 (59)	32 (51)	28 (67)
$Watt_{max}$, W	111 (83, 140)	127 (98, 170)	130 (95, 163)	124 (95, 170)	130 (85, 180)
$Watt_{max}$, % predicted (23)	77 ± 17	86 ± 22	85 ± 25	86 ± 21	78 ± 20
$Watt_{max}$, % predicted* (21, 23)	79 ± 18	92 ± 24	91 ± 27	92 ± 22	87 ± 20
$Watt_{max}$, <93% predicted, *n* (%)	27 (84)	358 (65)	23 (59)	44 (70)	31 (74)
HR_{max}, % predicted	92 (86, 96)	93 (88, 97)	91 (87, 97)	92 (87, 96)	92 (86, 96)
Respiratory exchange ratio	1.18 (1.12, 1.25)	1.16 (1.10, 1.23)	1.16 (1.11, 1.24)	1.14 (1.09, 1.20)	1.17 (1.08, 1.23)
$\dot{V}E_{peak}/MVV_{pred}$, %*	81 (72, 106)	88 (71, 103)	95 (72, 118)	75 (64, 92)	80 (61, 101)

Definition of abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator; FEV_1 = forced expiratory volume in 1 second; HR_{max} = maximum heart rate; MVV_{pred} = predicted maximum voluntary ventilation (calculated as $FEV_1 \times 35$); $\dot{V}E_{peak}$ = peak minute ventilation; $\dot{V}O_{2peak}$ = peak oxygen consumption; $Watt_{max}$ = maximum work rate.

Data are mean ± SD, median (interquartile range), or number (percent). Percent predicted values for $\dot{V}O_{2peak}$ were calculated using equations from Orenstein (22) and Jones and colleagues (21). Data for $\dot{V}O_{2peak}$ are shown separately according to Orenstein (22) and a combination of Orenstein (22) and Jones and colleagues (21) for patients aged 16 years or younger and patients older than 16 years of age, respectively. $Watt_{max}$ percent predicted values were calculated using equations from Godfrey and colleagues (23) and Jones and colleagues (21). Data are shown separately according to Godfrey and colleagues (23) and a combination of Godfrey and colleagues (23) and Jones and colleagues (21) for patients aged 16 years or younger and patients older than 16 years of age, respectively. Reduced exercise capacity ($\dot{V}O_{2peak}$ <82% predicted and $Watt_{max}$ <93% predicted) was calculated according to Nixon and colleagues (10). Statistical comparisons between different CFTR classes were performed using analysis of variance, a nonparametric Kruskal–Wallis test, or the χ^2 test for categorical variables.

*$P < 0.05$, significantly different between CFTR classes.

†$P < 0.001$, significantly different between CFTR classes.

potential underlying molecular mechanisms of the CFTR defect and its potential consequences on peripheral muscle function and exercise capacity. Recently, the expression of functional CFTR channels in human skeletal muscle was demonstrated (3, 4). Lamhonwah and colleagues (4) speculated that a dysfunction in sarcoplasmic reticulum CFTR channels might affect calcium release and thus impact the adenosine triphosphate–mediated actin–myosin interaction that is essential for muscle contraction. Moreover, Divangahi and colleagues (3) suggested that excessive systemic inflammation initiates a process in which the abnormal vulnerability of CFTR-deficient muscle to proinflammatory mediators could play a key role in the development of skeletal muscle weakness observed in individuals with CF (7). Of note, these observations are based on animal experiments and have not yet been confirmed in humans. On the basis of the *in vitro* experiments suggesting that systemic inflammation in combination with CFTR dysfunction may impair skeletal muscle function, it is reasonable to speculate that potential detrimental effects of *CFTR* genotype on maximal exercise capacity would be detected only with high levels of inflammation. However, although the present study included patients with mildly impaired pulmonary function (mean ± SD FEV_1, 77.3 ± 23% predicted), a subgroup analysis including only patients with moderate to severe lung disease (FEV_1, ⩽60% predicted) yielded similar results for the comparison between minimal function and residual function mutations (Tables E5 and E6). However, this subanalysis is limited by the small sample size of patients with residual function mutations and should be interpreted with caution. It remains to be shown in larger (ideally prospective) studies whether our findings are applicable to patients with more advanced disease and substantial inflammation.

Our main aim was to compare maximal exercise capacity between patients with CF carrying only minimal function *CFTR* mutations (classes I–III) and those with at least one residual function (class IV or V) *CFTR* mutation. In the primary analysis, our data showed no differences in maximal exercise capacity between groups in both unadjusted and adjusted analyses. These results were confirmed by the use of allometric models that were computed to exclude potential limitations of the prediction

Table 3. Clinical characteristics and cardiopulmonary exercise testing data between patients with two *CFTR* mutations in class I, II, or III compared with patients with at least one mutation in class IV or V

Variable	CFTR Classes I–III	CFTR Classes IV–V
No. of patients	621	105
CFTR modulator therapy, *n* (%)	9 (1)	3 (3)
Age, yr	16.2 (12.9, 21.6)	18.0 (13.0, 25.5)
Sex, % female	273 (44)	55 (52)
Cystic fibrosis–related diabetes, *n* (%)*	75 (12)	3 (3)
Pancreatic insufficiency, *n* (%)*	585 (95)	25 (24)
Pseudomonas aeruginosa, *n* (%)†	332 (54)	38 (36)
Body mass index, *z*-score*	−0.25 (−0.95, 0.42)	−0.11 (−0.77, 0.74)
Body fat, %†	18.2 ± 5.7	21.8 ± 6.4
Lean body mass, kg	41.1 (32.5, 50.3)	44.1 (36.4, 54.0)
FEV_1, % predicted	79 (59, 93)	84 (68, 96)
$\dot{V}O_{2peak}$, L/min	1.74 (1.4, 2.2)	1.78 (1.4, 2.4)
$\dot{V}O_{2peak}$, % predicted (22)	79 ± 19	80 ± 19
$\dot{V}O_{2peak}$, % predicted (21, 22)	80 ± 19	82 ± 20
$Watt_{max}$, W	125 (95, 168)	130 (94, 176)
$Watt_{max}$, % predicted (23)	86 ± 22	83 ± 20
$Watt_{max}$, % predicted (21, 23)	91 ± 24	90 ± 21
HR_{max}, % predicted	93 (88, 96)	92 (87, 96)
Respiratory exchange ratio	1.16 (1.10, 1.23)	1.15 (1.09, 1.23)
$\dot{V}E_{peak}/MVV_{pred}$, %*	88 (71, 104)	78 (63, 96)

Definition of abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator; FEV_1 = forced expiratory volume in 1 second; HR_{max} = maximum heart rate; MVV_{pred} = predicted maximum voluntary ventilation (calculated as $FEV_1 \times 35$); $\dot{V}E_{peak}$ = peak minute ventilation; $\dot{V}O_{2peak}$ = peak oxygen consumption; $Watt_{max}$ = maximum work rate.

Data are mean ± SD, median (interquartile range), or number (percent). Percent predicted values for $\dot{V}O_{2peak}$ were calculated using equations from Orenstein (22) and Jones and colleagues (21). Data for $\dot{V}O_{2peak}$ are shown separately according to Orenstein (22) and a combination of Orenstein (22) and Jones and colleagues (21) for patients aged 16 years or younger and patients older than 16 years of age, respectively. $Watt_{max}$ percent predicted values were calculated using equations from Godfrey and colleagues (23) and Jones and colleagues (21). Data are shown separately according to Godfrey and colleagues (23) and a combination of Godfrey and colleagues (23) and Jones and colleagues (21) for patients aged 16 years or younger and patients older than 16 years of age, respectively. Statistical comparisons between the two groups were performed using an independent *t* test, a nonparametric Mann-Whitney *U* test, or the χ^2 test, as appropriate.

*$P < 0.05$, significantly different between CFTR classes.

†$P < 0.001$, significantly different between CFTR classes.

equations for $\dot{V}O_{2peak}$ and $Watt_{max}$ (32). Regardless of the analytic approach, including adjustments for important confounders, we found robust evidence that *CFTR* genotype severity was not related to impaired maximal exercise capacity in patients with CF.

Our findings support previous studies showing that pulmonary function limitation and inadequate nutrition contribute to exercise intolerance in patients with CF (9, 13, 29, 30, 33, 34). Nutritional status plays a critical role in the progression of lung disease. Compared with healthy subjects, patients with CF exhibit peripheral muscle weakness (35) that is associated with reduced aerobic exercise capacity (7, 8). Thus, adequate nutrition and maintenance of BMI, and in particular muscle mass (35), are important for preserving exercise tolerance in patients with CF. The strong impact of lung function and nutritional status could mask the effect of *CFTR* genotype on exercise capacity in patients with CF. Consequently, to exclusively study the role of *CFTR* genotype on exercise capacity, we performed a subanalysis restricted to patients with preserved lung function (FEV_1, ⩾80% predicted) and nutritional status (BMI *z*-score, ⩾50th percentile), and the results remained qualitatively the same.

Our data are in contrast to a study by Selvadurai and colleagues (12), who reported a relationship between the severity of CFTR functional impairment and reduced exercise capacity using univariate analysis in children with CF aged 8–17 years with at least one copy of the *F508del-CFTR* mutation. In univariate analysis, we noticed no differences between groups in either absolute or percent predicted values for $\dot{V}O_{2peak}$ or $Watt_{max}$ (Table E6). However, in the adjusted analysis, patients with one copy of a class V mutation had significantly lower exercise capacity (about 8% predicted for both $\dot{V}O_{2peak}$ and $Watt_{max}$) than patients homozygous for the *F508del-CFTR* mutation (Tables E7 and E8). Patients with one copy of a class V mutation did not differ in ethnicity and lung function but had better nutritional status compared with patients homozygous for the *F508del-CFTR* mutation. However, there was no evidence for ventilatory limitation (i.e., lower breathing reserve) during exercise in these patients, which may suggest that the lower exercise performance was reflective more of fitness level than of pulmonary status. Moreover, in mixed models, the presence of *P. aeruginosa* infection was significantly associated with a lower $\dot{V}O_{2peak}$ and $Watt_{max}$ (Table E8). This is supported by a study by van de Weert–van Leeuwen and colleagues (13), who have previously shown that the presence of *P. aeruginosa* in adolescents with CF is a predictor of a steeper decline in $\dot{V}O_{2peak}$ over time, independent of age, nutritional status, pulmonary function, and immunoglobulin G levels. Nevertheless, relatively fewer patients with a group V mutation were colonized with *P. aeruginosa* than patients with solely *CFTR* class II mutations (reference group), despite a lower $\dot{V}O_{2peak}$ in the former than in the latter group. The differences between our study and the study by Selvadurai and colleagues (12) might be explained by the preserved lung function in the present cohort of patients and our rigorous adjustment for important clinical confounders in the statistical models. In addition, substantial *CFTR* genotype misclassification affecting different groups in the study by Selvadurai and colleagues (12) may contribute to the diverse findings. Finally, we can only speculate that lower habitual physical activity, a determinant of $\dot{V}O_{2peak}$ in CF (9), contributes to the reduced exercise capacity in patients with one copy of a class V mutation. The cohort of patients with a class IV or V *CFTR* mutation in our study might be a subgroup of patients that have been diagnosed at older ages (i.e., when their CF disease progressed and started to become symptomatic). Thus, later CF diagnosis and less focus clinically on exercise and physical activity may affect

Table 4. Mixed models for association between patients with two *CFTR* mutations in class I, II, or III (group I–III) compared with patients with at least one mutation in class IV or V (group IV–V)

	β-Coefficient (95% CI)	SE	*P* Value
$\dot{V}O_{2peak}$, % predicted			
Age	−0.14 (−0.32 to 0.04)	0.09	0.14
Sex	−1.29 (−3.46 to 0.88)	1.11	0.24
Pseudomonas aeruginosa	−1.93 (−4.44 to 0.58)	1.28	0.13
Body mass index, *z*-score	1.78 (0.78 to 2.77)	0.51	<0.001
FEV_1, % predicted	0.41 (0.35 to 0.47)	0.03	<0.001
CFTR group	−0.95 (−4.18 to 2.29)	1.65	0.57
$Watt_{max}$, % predicted			
Age	0.02 (−0.19 to 0.23)	0.11	0.87
Sex	0.23 (−2.21 to 2.66)	1.24	0.85
Pseudomonas aeruginosa	−2.85 (−5.69 to −0.22)	1.45	0.048
Body mass index, *z*-score	1.30 (0.19 to 2.42)	0.57	0.02
FEV_1, % predicted	0.46 (0.40 to 0.53)	0.03	<0.001
CFTR group	−1.38 (−5.04 to 2.27)	1.86	0.46

Definition of abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator; CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; $\dot{V}O_{2peak}$ = peak oxygen consumption, $Watt_{max}$ = maximum work load.
The categorical variable *Pseudomonas aeruginosa* is coded as 0 = no and 1 = yes. Sex is coded as 0 for females and 1 for males. CFTR groups are coded as 0 (classes I–III combined) and 1 (classes IV–V combined). $\dot{V}O_{2peak}$ and $Watt_{max}$ percent predicted values were calculated using reference equations from Orenstein (22) and Godfrey and colleagues (23), respectively.

behavior in early childhood leading to a less active lifestyle.

It is important to note that $\dot{V}O_{2peak}$ shows a large variation across the population, and available prediction equations have their limitations. Most of the equations that are used in CF research were established several decades ago, and mostly on the basis of small study samples that did not cover the whole age range of our population and/or did not consider both sexes (21, 22, 36). We decided to use $\dot{V}O_{2peak}$ prediction equations published by Orenstein (22) because these equations were shown to be related to health-related quality of life (37), patient-reported health status (38), and mortality (10) in patients with CF. In addition, we tested another frequently used prediction equation for $\dot{V}O_{2peak}$ (21), and the results of all models were comparable to the prediction equation published by Orenstein (22). However, when using equations from Jones and colleagues (21), we noticed significant associations between $\dot{V}O_{2peak}$ (and $Watt_{max}$ in percent predicted) with age and sex in the adjusted random effects models, which raises concern about the validity of these equations for this specific CF population.

This study has several limitations. First, we collected data from different international study centers. Thus, we cannot rule out differences in treatment strategies and treatment quality possibly affecting the health status of the patients, thereby introducing bias. Second, the groups classified according to CFTR classes were unevenly distributed, with a high number of patients in the group with the most common *F508del-CFTR* mutation (39, 40) compared with patients carrying a *CFTR* class I, III, IV, or V mutation. Despite the large sample size of 726 patients from 17 different CF centers worldwide, group sizes with *CFTR* classes I and III–V mutations were relatively small owing to the generally low prevalence of these gene mutations (41). For these reasons, the exploratory analysis comparing exercise capacity among patients with at least one copy of the *F508del-CFTR* mutation should be interpreted with caution. Moreover, we were not able to consider all known confounders impacting exercise capacity, such as physical activity (9), inflammatory markers, and other CF airway pathogens that were either not routinely assessed or not available for this retrospective analysis. Nevertheless, strong predictors of $\dot{V}O_{2peak}$, such as pulmonary function (9, 13, 29, 30, 33) and nutritional status (34), as well as proxy measures of inflammation (i.e., *P. aeruginosa* status), were included in our statistical analysis. In addition, our analysis was limited to maximal CPET outcomes. Additional measures such as $\dot{V}O_2$ at the anaerobic threshold would have provided further insight into muscle (dys) function. Finally, we acknowledge the limitation of the retrospective study design and the collection of data over a large time period. However, it seems practically impossible to acquire such a large dataset on CPET variables within a prospective study that would overcome these limitations.

In summary, in our large, international cohort of children, adolescents, and adults with CF, we did not detect an association between *CFTR* genotype group and maximal exercise capacity. In this cohort, lower pulmonary function and worse nutritional status were associated with reduced exercise capacity. These findings underline the importance of preserving lung function and maintaining adequate nutrition to prevent exercise intolerance in patients with CF. ■

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