Sleep Phase Delay in Cystic Fibrosis

A Potential New Manifestation of Cystic Fibrosis Transmembrane Regulator Dysfunction

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BACKGROUND: Cystic fibrosis (CF) transmembrane regulator (CFTR) protein dysfunction causes CF. Improving survival allows detection of increasingly subtle disease manifestations. *CFTR* dysfunction in the central nervous system (CNS) may disturb circadian rhythm and thus sleep phase. We studied sleep in adults to better understand potential CNS *CFTR* dysfunction.

METHODS: We recruited participants from April 2012 through April 2015 and administered the Munich Chronotype Questionnaire (MCTQ). We compared free-day sleep measurements between CF and non-CF participants and investigated associations with CF survival predictors.

RESULTS: We recruited 23 female and 22 male adults with CF aged 18 to 46 years and 26 female and 22 male volunteers aged 18 to 45 years. Compared with volunteers without CF, patients with CF had delayed sleep onset (0.612 h; $P = .015$), midsleep (1.11 h; $P < .001$), and wake (1.15 h; $P < .001$) times and prolonged sleep latency (7.21 min; $P = .05$) and duration (0.489 h; $P = .05$). Every hour delay in sleep onset was associated with shorter sleep duration by 0.29 h in patients with CF and 0.75 h in subjects without CF ($P = .007$) and longer sleep latency by 7.51 min in patients with CF and 1.6 min in volunteers without CF ($P = .035$). Among patients with CF, FEV₁ % predicted, prior acute pulmonary exacerbations, and weight were independent of all free-day sleep measurements.

CONCLUSIONS: CF in adults is associated with marked delays in sleep phase consistent with circadian rhythm phase delays. Independence from disease characteristics predictive of survival suggests that sleep phase delay is a primary manifestation of *CFTR* dysfunction in the CNS.

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ABBREVIATIONS: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane regulator; MCTQ = Munich Chronotype Questionnaire

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Steady progress in treatment continues the remarkable improvements in survival¹ observed since the modern description of cystic fibrosis (CF).^{2,3} CF transmembrane regulator (*CFTR*) gene sequencing demonstrated that *CFTR* protein dysfunction underlies clinical disease.⁴⁻⁶ Progressive pulmonary and GI diseases characterize classic CF disease observed over centuries³ and cause early mortality.^{1,7} Increased survival time, milder disease, and knowledge of a specific, disease-causing, abnormal or absent protein facilitate bench and clinical investigations that expand the list of outcomes directly attributable to *CFTR* dysfunction. Recent development of mutation-specific modulators of *CFTR* protein synthesis⁸⁻¹² suggests the

possibility of directly treating new primary manifestations.

Defective *CFTR* expression in the brain may lead to detectable abnormalities.¹³⁻¹⁵ Dysfunctional *CFTR* protein in the retina^{16,17} and anterior hypothalamus¹⁸⁻²⁰ may phase shift and reduce the adaptability of the master circadian clock,²¹ adversely affecting health,²²⁻²⁷ social functioning,²⁸⁻³¹ and quality of life.^{22,32-35} Because sleep phase delay is commonly associated with circadian phase delay, we performed a pilot study of sleep phase using noninvasive low-cost, but effective, tools. We hypothesize that sleep phase delay may be a direct clinical manifestation of *CFTR* dysfunction in the CNS.

Methods

Participants

With University of Utah Investigational Review Board approval (IRB_00005311) and informed consent, we serially recruited adult patients with CF during outpatient visits or hospitalizations for acute pulmonary exacerbations. We recorded age, height, weight, sex, race, ethnicity, CF genotype, and FEV₁.³⁶⁻³⁸ We normalized FEV₁ to FEV₁ % predicted using National Health And Nutrition Examination Survey (NHANES) III³⁹ and Global Lung Initiative (GLI) equations.⁴⁰ We counted acute pulmonary exacerbations requiring hospitalization in the year prior to study enrollment. At our institution, we diagnose acute pulmonary exacerbations and hospitalize for at least one symptom and one objective finding of acutely worsened lung disease.⁴¹ We do not hospitalize patients with increased symptoms only or objective findings only and did not count those episodes as exacerbations.

We recruited volunteers without CF from the University of Utah Medical Center and local communities. We collected age, height, sex, race, and ethnicity and deidentified the data on collection.

Sleep Phase Evaluation

Till Roenneberg provided permission to use the Munich Chronotype Questionnaire (MCTQ)⁴² for research purposes (personal e-mail communication, March 22, 2012 with reconfirmation February 10, 2017). All participants completed the unaltered latest version of the MCTQ,⁴² which asks for information concerning bedtime, sleep-onset, and wake times and minutes needed to fall asleep on nights before work and on free days. A lack of obligations requiring specific wake times defines free days.⁴³ To prevent confusion, we taught participants the definitions used by Dr Roenneberg⁴²: (1) sleep-onset time is the time a participant gets into bed (bedtime) plus the time needed to fall asleep (sleep latency) and (2) wake time is the time that a

participant becomes awake and not the time that a participant gets out of bed.

Statistical Analysis

We calculated summary statistics. Using sleep-onset and wake times, we estimated midsleep time (the standard single clock time that describes the extent of sleep phase delay or advancement and best correlates with dim light melatonin onset or onset of true biological night)⁴⁴ and sleep duration (the maximal possible total time asleep from sleep onset to wake time) on the night prior to a free day.⁴³ We compared basic characteristics between patients and volunteers using *t* tests⁴⁵ and compared sleep onset, midsleep, and wake times as well as sleep latency and duration using linear regression. Using multivariable linear regression adjusted for age, sex, and height, we performed backward selection based on Wald test *P* values of each explanatory variable to determine the most parsimonious model for each of the sleep measurements. We performed multivariable linear regressions of each of the five sleep phase measurements, with sleep-onset time as the independent variable of main interest adjusted for age, sex, and height with backward selection. With models involving only patients with CF, we performed forward selection with FEV₁ % predicted, number of acute pulmonary exacerbations in the year prior to administration of the MCTQ, weight, height, and F508del homozygous status to explore relationships between the main characteristics of CF that predict survival⁷ and sleep phase. With each selection step in each multivariable model, we examined the stability of coefficients, SEs, and *P* values. For models with acute pulmonary exacerbations as the outcome, we used quasi-Poisson regression because the data were not clearly Poisson in dispersion. Because all participants live in the Salt Lake City area and participated during spring, summer, and fall when daylight hours are longest in Utah, we did not adjust for latitude, longitude, or MCTQ administration date. We used the R statistical system for all analyses.⁴⁶

Results

Participants

We recruited 47 patients with CF and 50 volunteers without CF. We discarded two questionnaires from

patients and two from volunteers because of incompleteness. Study groups had the same frequencies regarding sex, race, and ethnicity. All were young adults, but there were small differences in distribution of age and height (Table 1, e-Fig 1). All participants were white

TABLE 1] Demographics of Study Participants

Characteristic	Patients With CF (n = 45)	Control Volunteers Without CF (n = 48)	P Value
Female sex, No. (%)	23 (51)	26 (54)	NS
Age	29.6 (6.8; 19.0-47.0)	27.4 (6.7, 18.0-45.0)	.007
Height, cm	168.1 (9.5; 152.0-187.4)	174.3 (9.0, 158.0-190.0)	.002
Weight, kg	62.3 (14.5; 37.9-98.0)	...	...
FEV ₁ % predicted (NHANES III)	62.0 (23.8; 19.0-105.0)	...	...
FEV ₁ % predicted (GLI)	62.1 (23.8; 18.9-104.2)	...	...
No. of prior acute pulmonary exacerbations	2.2 (2.7; 0-14)	...	...
No. of F508del homozygotes, No. (%)	24 (53)	...	...

Data are presented as mean values (SD, range) except as noted. GLI = Global Lung Initiative; NHANES III = National Health And Nutrition Examination Survey III; NS = not significant.

except one Hispanic female with CF, consistent with the racial distribution of CF in Utah.

Sleep Analyses

Univariable linear regressions showed that patients with CF had significantly later sleep onset and midsleep and wake times and longer sleep latency and duration than did volunteers without CF (Table 2). Multivariable linear regressions adjusted for age, sex, and height gave similar estimates (Table 3).

Independent of CF diagnosis, increasing age significantly moved sleep onset, midsleep, and wake times to earlier in the night while lengthening average sleep latency (e-Table 1A-D) but had no significant effect on sleep duration. Female subjects had a later sleep onset (by 53 minutes) (e-Table 1A) and a shorter sleep duration (by 42 minutes) (e-Table 1E) on average. Greater height was significantly associated only with later time of midsleep (e-Table 1B).

We evaluated variation among subjects that was associated with the choice of earlier or later bedtimes. For every hour sleep onset was later (Table 4), patients

with CF had a 7.51-min prolongation in sleep latency (95% CI, 3.75 min; 11.27 min) and nearly an hour delay in both midsleep (95% CI, 0.74 h; 0.97 h) and wake times (95% CI, 0.48 h; 0.93 h), but the total duration of sleep was shorter by about 18 min (95% CI, -3.76 min; -31.28 min). Adjustment by patient age was significantly associated with sleep latency, midsleep, and wake times and sleep duration (e-Table 2A-D). In contrast, a 1-h delay in sleep onset in volunteers without CF was not associated with sleep latency or wake time but was associated with a midsleep time 38 min later on average (95% CI, 22.84 min; 40.27 min) and a sleep duration that was shorter by 45 min (95% CI, -62.40 min; -27.60 min). Age adjustments had no significant effect in volunteers without CF, and sex and height adjustments had no significant effects for either patients with CF or volunteers without CF.

Sleep latency effects were prolonged and other sleep effects were decreased with each additional year of age (e-Table 2). Midsleep and wake times and sleep latency

TABLE 2] Sleep Phase Delay Due to CF: Univariable Models

Sleep Measure	Estimate ^a	SE ^a	P Value
Sleep-onset time	0.547	0.263	.04
Midsleep time	0.781	0.243	.002
Wake time	1.01	0.288	< .001
Sleep latency (min)	8.610	3.670	.02
Sleep duration	0.467	0.259	.07

CF = cystic fibrosis.

^aSleep measure estimates and SE are in hours except as noted.

TABLE 3] Sleep Phase Delay Due to CF: Multivariable Models Adjusted by Age, Sex, and Height^a

Sleep Measure	Estimate ^a	SE ^a	P Value
Sleep-onset time	0.612	0.247	.015
Midsleep time	1.110	0.246	< .001
Wake time	1.150	0.279	< .001
Sleep latency (min)	7.210	3.630	.05
Sleep duration	0.489	0.250	.05

Estimates and SE are in hours except as noted. See Table 2 legend for expansion of abbreviations.

^aEach estimate is the effect of CF as an explanatory variable adjusted for age, sex, and height when statistically significant. The estimates of effects for statistically significant adjustment coefficients are shown in e-Table 1.

TABLE 4] Sleep Phase Delay Associated With Later Sleep-Onset Times in CF

Participants	Sleep Outcome Affected by Later Sleep-Onset Times	Estimate ^a	SE	P Value
Patients with CF	Sleep latency (min)	7.510	1.920	< .001
	Midsleep time	0.854	0.0584	< .001
	Wake time	0.708	0.117	< .001
	Sleep duration	-0.292	0.117	.016
Volunteers without CF	Midsleep time	0.625	0.0741	< .001
	Sleep duration	-0.750	0.148	< .001

See Table 2 legend for expansion of abbreviations.

^aEstimates for patients with CF are for the effects of later sleep-onset times adjusted for age; sex and height adjustments were not significant. Estimates of effects of age are shown in e-Table 2. Estimates for effects of later sleep-onset times in volunteers without CF are unadjusted because age, sex, and height were not significant.

and duration associated with later sleep onset were not significantly associated with sex or height.

Using either linear or quasi-Poisson regression, as appropriate, we found no significant relationships between sleep latency or duration or sleep-onset, midsleep, or wake times with FEV₁ % predicted, number of acute pulmonary exacerbations in the year prior to administration of the MCTQ, or weight (Fig 1) in patients with CF. There were no significant associations with F508del homozygous state. Using univariable quasi-Poisson regression, we found that the number of acute pulmonary exacerbations was strongly associated with lung disease severity in patients with CF (Fig 2). Multivariable models adjusted for age, sex, and height failed to uncover significant associations between any sleep measurements and FEV₁ % predicted, number of acute pulmonary exacerbations, or weight. Using NHANES III or GLI equations for normalization of FEV₁ produced nearly identical values of FEV₁ % predicted (e-Fig 2, e-Table 3) with no effect on results (Table 1, e-Figs 3, 4).

Discussion

We performed a pilot study examining sleep timing and duration among patients with CF compared with volunteers without CF. We found that adults with CF have markedly delayed sleep phase and prolonged sleep latency and duration (Table 2). Study patients had a wide range of disease, from normal lung function and no acute pulmonary exacerbations in the year prior to MCTQ administration to an FEV₁ approximately 20% of predicted and more than five exacerbations (Fig 2), providing the opportunity to detect relationships with measurements of sleep phase. In fact, we found no relationship between any sleep phase measurement and FEV₁ % predicted, number of prior acute pulmonary exacerbations, or weight (Fig 1, e-Fig 3), the three major

determinants of survival in CF.⁷ The marked differences in sleep phase measurements among patients with CF compared with control volunteers without CF (Table 3) support the possibility that CF is associated with circadian rhythm delay, and the lack of significant associations with disease severity markers is consistent with the possibility that sleep phase delay is a novel clinical manifestation of circadian cycle abnormality due to *CFTR* dysfunction in the CNS.²¹

Later sleep onset was associated with longer sleep latency and later wake times in patients with CF, relationships not seen in volunteers without CF (Table 4). All study participants had decreased sleep duration associated with later sleep onset; however, the effect was twice as large in volunteers. The differences between our study groups underscore the marked differences in sleep phase associated with CF; however, these findings cannot distinguish between an effect due to the clinical biology of *CFTR* dysfunction or differing societal influences associated with chronic CF disease.

Sleep findings were stable after adjustments for age, sex and height. After selection procedures, most of the multivariable models retained age as a significant covariate (e-Tables 1A-D, 2A-D). Some models retained female sex (e-Table 1A, 1E) and increasing height (e-Table 1B). Similar age-, sex-, and height-related effects have been described and provide reassurance that results derived from the control volunteers without CF are consistent with prior large population studies of sleep, particularly those using the MCTQ,³⁷ and provide a reasonable basis for comparison to discover CF-specific differences in sleep phase.

A circadian cycle abnormality may be a simple phase shift that causes later bedtimes and wake times relative to a typical circadian cycle.⁴⁷ More complex circadian

Figure 1 – Sleep phase measurements and clinical data for patients with cystic fibrosis (CF). A, We found no relationship between lung function, prior acute pulmonary exacerbations, or weight with sleep latency or total sleep duration in patients with CF. Compared with control subjects, patients with CF had longer sleep latency that was significant without adjustment and prolonged sleep duration that was borderline in significance (Table 2). However, both latency and duration were significantly longer in patients with CF after adjustment for age, sex, and height (Table 3). B, Timing of sleep onset, midsleep, and wake times were independent of FEV₁ % predicted, number of acute pulmonary exacerbations in the prior year, and weight among patients with CF. Volunteers without CF had earlier times for all three sleep events. The differences were significant without adjustment (Table 2), and the associations were strengthened after adjustments for age, sex, and height, as appropriate (Table 3, e-Table 1A-C). APE = acute pulmonary exacerbation; CF = cystic fibrosis.

abnormalities may include non-24-hour cycles or cycles that are associated with irregular sleep-wake patterns. Determination of the circadian cycle abnormality types that patients with CF may have will require more extensive methods than used in the current study.

A circadian phase shift and accompanying sleep phase delay need not cause an intrinsic physiological abnormality. However, such a phase delay interferes with daily life activities that begin in the morning, for example, 8 AM start times for school or work. Individuals

with severe phase delays may experience academic failures^{48,49} because of the inability to attend and perform well in morning classes. These individuals may be unable to hold jobs except those that start in the afternoon and continue through the evening or night, a circumstance that may lead to economic consequences or limit social interactions with friends or families in afternoon or evening settings. Individuals with sleep phase delay who try to work typical daytime hours may experience a form of shift work disorder that can lead to a variety of physical ailments.^{31,32,50,51}

Figure 2 – Relationship between prior acute pulmonary exacerbations and FEV₁ % predicted. FEV₁ % predicted, normalized using National Health And Nutrition Examination Survey III equations, and the number of acute pulmonary exacerbations in the prior year (truncated to a maximum of five) are the two most important predictors of survival in CF.⁷ Patients with CF who participated in this study had a wide range of FEV₁ % predicted values and number of prior acute pulmonary exacerbations requiring hospitalization. Using quasi-Poisson regression, we detected a strong inverse relationship. Note that acute pulmonary exacerbation counts were jittered to better show individual values. See Figure 1 legend for expansion of abbreviations.

Our study was a pilot and thus subject to potential shortcomings. We used serial recruitment and thus might have enrolled a nonrepresentative patient population with CF, resulting in poor generalizability of our results. However, the wide ranges of FEV₁ % predicted and prior acute pulmonary exacerbation counts (Fig 2) and the broad range of sleep phase findings (Tables 2-4) provide reassurance that the lack of significant associations was not due to a lack of variation in severity of measurements. We performed analyses twice involving FEV₁ % predicted normalized with NHANES III³⁹ and GLI equations.⁴⁰ Results were similar (Table 1, e-Figs 2-4, e-Table 3) showing that for the adults with CF, the choice of equation had no impact. The number of study participants included > 20% of our adult CF center population, suggesting that our results were not spurious because of a few unusual individuals. We might have undercounted acute pulmonary exacerbations if care was obtained outside our institution. However, most hospitalizations elsewhere result in transfers to our CF center, the only one accredited for adults in Utah. We internally audit charts to prevent underreporting of pulmonary

exacerbations, although we cannot exclude the possibility that some patients avoid care altogether when ill. Readers should note that we define acute pulmonary exacerbations of CF in a way that depends only on data available at the time of evaluation to facilitate prospective application at the point of care.⁴¹ That definition does not require calculating pulmonary exacerbation or pulmonary exacerbation risk scores^{52,53} nor does it consider any retrospective criterion⁵⁴ that may affect the interpretation of our results.

Our evaluation of sleep phase changes relative to a control group of volunteers without CF controlled for societal behaviors regarding sleep patterns. We did not, however, explore differences in the use of electronic devices that allow social media interactions while adhering to infection control guidelines. These devices may produce blue light, which can phase shift the circadian clock.⁵⁵ Future investigation might consider whether patients with CF use these devices more frequently than do the volunteers without CF. There were minor differences in the demographics of control subjects compared with study patients. Control volunteers were younger and taller (Table 1). However, absolute differences were small, were adjusted for in multivariable analysis, and ultimately had no impact on results or their interpretation.

We did not measure salivary melatonin levels or ask participants to wear actigraphs to ascertain the onset and duration of biological night²² in the study population due to the cost of measurements and equipment and the 6-h time requirement to collect samples.^{34,56,57} Actigraph and additional sleep questionnaire data might confirm that coughing and poor sleep quality do not explain sleep phase delay.⁵⁸

Nevertheless, our primary goal was to generate sufficient evidence to support the overall hypothesis that sleep phase delay may be a direct clinical manifestation of *CFTR* dysfunction in the CNS. Treating such a sleep phase delay might substantially improve health and well-being. Our study accomplished this goal and provides the data necessary to plan a more extensive and thorough future investigation, perhaps involving a multicenter design with melatonin measurements and actigraph recordings along with a more extensive assessment of the impact of sleep phase delay on quality of life in patients with CF.

This study shows that sleep phase delay is common and severe in patients with CF. These preliminary findings already suggest potential low-risk accommodations such as afternoon appointments or medication schedules that

may improve adherence to clinic visits and treatments for patients having difficulty with early wake times. The evidence presented supports further investigation to better understand biological underpinnings of the clinical findings and societal functioning and quality of life. Confirmation of sleep phase delay with a better

understanding of subsequent clinical impacts would reveal a new manifestation of *CFTR* dysfunction that itself could be a new target for treatment, particularly among patients with normal or minimally diseased lungs who currently lack a formal indication for *CFTR* modulator treatment.

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Additional information: The e-Figures and e-Tables can be found in the Supplemental Materials section of the online article.

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