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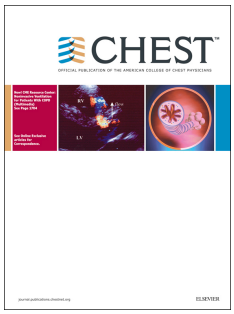

Sleep Phase Delay in Cystic Fibrosis: A Potential New Manifestation of Cystic Fibrosis Transmembrane Regulator Dysfunction

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Abbreviations

CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Regulator
CNS	Central Nervous System
MCTQ	Munich Chronotype Questionnaire
PEx	Pulmonary Exacerbations

Abstract:

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 18-46 years and 26 female and 22 male volunteers aged 18-45 years. Compared to non-CF volunteers, patients with CF had delayed sleep-onset (0.612 hrs, $P = 0.015$), mid-sleep (1.11 hrs, $P < 0.001$) and wake-up (1.15 hrs, $P < 0.001$) times and prolonged sleep latency (7.21 min, $P = 0.05$) and duration (0.489 hr, $P = 0.05$). Every hour delay in sleep-onset time was associated with shorter sleep duration by 0.29 hours in CF and 0.75 hours in non-CF ($P = 0.007$) and longer sleep latency by 0.15 hours in CF and 0.05 in non-CF ($P = 0.035$). Among patients with CF, FEV_1%, prior acute pulmonary exacerbations and weight were independent of all free-day sleep measurements.

Conclusion: 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.

Steady progress in treatment continues the remarkable improvements in survival[1] 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 gastrointestinal disease characterize classic CF disease observed over centuries[3] 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-synthesis[8–12] 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 hypothalamus[18–20] may phase-shift and reduce 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 non-invasive, low cost but effective tools. We hypothesize that sleep phase delay may be a direct clinical manifestation of *CFTR* dysfunction in the central nervous system (CNS).

Materials and 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 forced expiratory volume in one second (FEV_1).[36–38] We normalized FEV_1 to percent predicted FEV_1 (FEV_1%) using National Health And Nutrition Examination Survey (NHANES) III[39] 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 only increased symptoms or only objective findings and did not count those episodes as exacerbations.

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

Sleep Phase Evaluation

Till Roenneberg provided permission to use the Munich Chronotype Questionnaire (MCTQ)[42] for research purposes (personal communication). All participants completed the unaltered latest version of the MCTQ[42] which asks for information concerning bed, sleep-onset and wake-up times and minutes needed to fall asleep for nights before work and free days. A lack of obligations requiring specific wake-up times defines free days.[43] To prevent confusion, we taught participants the definitions used by Dr. Roenneberg:[42] (1) sleep-onset time as the time a participant gets into bed (bed-time) plus the time needed to fall asleep (sleep latency), and (2) wake-up time as the time that a participant last becomes awake and not the time that a participant gets out of bed.

Statistical Analysis

We calculated summary statistics. Using sleep-onset and wake-up times, we estimated mid-sleep 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-up times) on the night prior to a free-day.[43] Between patients and volunteers, we compared basic characteristics using *t*-tests[45] and compared sleep-onset, mid-sleep and wake-up times and 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 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 FEV_1%, number of acute pulmonary exacerbations in the year prior to MCTQ administration, weight, height and F508del homozygous status to explore relationships between the main characteristics of CF that predict survival[7] and sleep phase. With each selection step in each multivariable model, we examined the stability of coefficients, standard errors 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 within 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 with regards to sex, race and ethnicity. All were young adults but there were small differences in distribution of age and height (Table 1, e-Figure 1). All participants were white except one Hispanic female with CF, consistent with the racial distribution of CF in Utah.

Sleep Analyses

Univariate linear regressions showed that patients with CF had significantly later sleep-onset, mid-sleep and wake-up times and longer sleep latency and duration than volunteers without

CF (Table 2A). Multivariable linear regressions adjusted for age, sex and height gave similar estimates (Table 2B).

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

We evaluated variation among subjects associated with the choice of earlier or later bed-times. For every hour later in sleep-onset time (Table 2C), patients with CF had a 7.51 minute prolongation in sleep latency (95% CI 3.75, 11.27 minutes) and nearly an hour delay in both mid-sleep (95% CI 0.74, 0.97 hours) and wake-up times (95% CI 0.48, 0.93 hours), but the total duration of sleep was shorter by about 18 minutes (95% CI = -3.76, -31.28 minutes). Adjustment by patient age was significantly associated with sleep latency, mid-sleep and wake-up times and sleep duration (e-Table 2A-D). In contrast, a one hour delay in sleep-onset time in volunteers without CF was not associated with sleep latency or wake-up time but was associated with a mid-sleep time on average 38 minutes later (95% CI = 22.84, 40.27 minutes) and sleep duration shorter by 45 minutes (95% CI = -62.40, -27.60 minutes). 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). Mid-sleep and wake-up times and sleep latency and duration associated with later sleep-onset times were not significantly associated with sex or height.

Using either linear or quasi-Poisson regression as appropriate, we found no significant relationships among patients with CF between sleep latency or duration or sleep-onset, mid-sleep or wake-up times with FEV_1%, number of acute pulmonary exacerbations in the year prior to MCTQ administration or weight (Figure 1). There were no significant associations with F508del

homozygous state. Using univariate quasi-Poisson regression, we found that among patients with CF, the number of acute pulmonary exacerbations was strongly associated with lung disease severity (Figure 2). Multivariable models adjusted for age, sex and height failed to uncover significant associations between any sleep measurements and FEV_1%, number of acute pulmonary exacerbations or weight. Using NHANES III or GLI equations for normalization of FEV_1 produced nearly identical values of FEV_1% (e-Table 3 and e-Figure 2) with no effect on results (Table 1 and supplemental e-Figures 3-4).

Discussion

We performed a pilot study examining sleep timings and durations 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 FEV_1 approximately 20% of predicted and more than 5 exacerbations (Figure 2) providing the opportunity to detect relationships with measurements of sleep phase. In fact, we found no relationship between any sleep phase measurement and FEV_1%, number of prior acute pulmonary exacerbations or weight (Figure 1 and e-Figure 3), the three major determinants of survival in CF.[7] The marked differences in sleep phase measurements among patients with CF compared to control volunteers without CF (Table 2B) 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 times were associated with longer sleep latency and later wake-up time in patients with CF, relationships not seen in volunteers without CF (Table 2C). All study participants had decreased sleep duration associated with later sleep-onset times, 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-Table 1A-D and e-Table 2A-D). Some models retained female sex (e-Table 1A and E) and increasing height (e-Table 1B). Similar age, sex and height-related effects have been described and provide reassurance that results derived from our 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 bed-times and wake-up times relative to a typical circadian cycle.[47] More complex circadian 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 employed in the current study.

A circadian phase shift and accompanying sleep phase delay need not cause an intrinsic physiologic 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 suffer 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 which 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 suffer a form of shift work disorder that can lead to a variety of physical ailments.[31,32,50,51]

Our study was a pilot and thus subject to potential shortcomings. We employed serial recruitment and thus might have enrolled a non-representative patient population with CF resulting

in poor generalizability of our results. However, the wide ranges of FEV_1% and prior acute pulmonary exacerbation counts (Figure 2) and the broad range of sleep phase findings (Table 2) reassure that the lack of significant associations was not due to a lack of variation in severity of measurements. We performed analyses twice involving FEV_1% normalized with NHANES III[39] and GLI equations.[40] Results were similar (Table 1, e-Table 3, e-Figures 2-4) showing that for our adults with CF, choice of equations had no impact. The number of study participants included over 20% of our adult CF Center population, suggesting that our results were not spurious because of a few unusual individuals. We might have under-counted acute pulmonary exacerbations if care was obtained outside of our institution. However, most hospitalizations elsewhere result in transfers to our CF center, the only accredited one for adults in Utah. We internally audit charts to prevent under-reporting 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 exacerbation to facilitate prospective application at the point-of-care.[41] That definition does not require calculating Pulmonary Exacerbation (PEx) or PEx Risk scores[52,53] nor consider any retrospective criterion[54] which 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 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 non-CF volunteers. There were minor differences in the demographics of controls compared to 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 night[22] within our study population due to the cost of

measurements and equipment and the six hour 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 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-up times. The evidence presented supports further investigation to better understand biological underpinnings of the clinical findings and better understand 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 lung who currently lack a formal indication for *CFTR* modulator treatment.

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Jensen and Dr Liou act as the guarantors of the paper and assume responsibility for the integrity of the work as a whole. Ms Jensen led and Ms Packer and Dr Liou assisted with initial presentation of preliminary data in August, 2015 at the Australasian CF Conference in Sydney, Australia. Dr Liou included preliminary data in a second presentation Dec 1, 2016 at Internal Medicine Grand Rounds at the University of Utah, Salt Lake City, UT, USA.

References

1. Cystic Fibrosis Foundation Patient Registry 2015 Annual Data Report [Internet]. Bethesda, Maryland: Cystic Fibrosis Foundation; 2016. Available from: http://www.cff.org/Our-Research/CF-Patient-Registry/

2. Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease: A clinical and pathologic study. *Am J Child* 1938;56(2):344–399.

3. Quinton PM. Physiological basis of cystic fibrosis: a historical perspective. *Physiol Rev* 1999;79(1 Suppl):S3–S22.

4. Kerem B, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;245(4922):1073–1080.

5. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245(4922):1066–1073.

6. Rommens JM, Iannuzzi MC, Kerem B, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;245(4922):1059–1065.

7. Liou TG, Adler FR, Fitzsimmons SC, Cahill BC, Hibbs JR, Marshall BC. Predictive 5-year survivorship model of cystic fibrosis. *Am J Epidemiol* 2001;153(4):345–52.

8. Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011;365(18):1663–1672.

9. Flume PA, Liou TG, Borowitz DS, et al. Ivacaftor in Subjects with Cystic Fibrosis who are Homozygous for the F508del-CFTR Mutation. *Chest* 2012;142(3):718–724.

10. Boyle MP, Bell SC, Konstan MW, et al. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. *Lancet Respir Med* 2014;2(7):527–538.

11. Elborn JS, Ramsey BW, Boyle MP, et al. Efficacy and safety of lumacaftor/ivacaftor combination therapy in patients with cystic fibrosis homozygous for Phe508del CFTR by pulmonary function subgroup: a pooled analysis. *Lancet Respir Med* 2016;4(8):617–626.

12. Mutyam V, Du M, Xue X, et al. Discovery of Clinically Approved Agents That Promote Suppression of Cystic Fibrosis Transmembrane Conductance Regulator Nonsense Mutations. *Am J Respir Crit Care Med* 2016;194(9):1092–1103.

13. Marcorelles P, Friocourt G, Uguen A, Ledé F, Férec C, Laquerrière A. Cystic fibrosis transmembrane conductance regulator protein (CFTR) expression in the developing human brain: comparative immunohistochemical study between patients with normal and mutated CFTR. *J Histochem Cytochem Off J Histochem Soc* 2014;62(11):791–801.

14. Mulberg AE, Resta LP, Wiedner EB, Altschuler SM, Jefferson DM, Broussard DL. Expression and localization of the cystic fibrosis transmembrane conductance regulator mRNA and its protein in rat brain. *J Clin Invest* 1995;96(1):646–652.

15. Reznikov LR. Cystic Fibrosis and the Nervous System. *Chest* 2016;

16. Blaug S, Quinn R, Quong J, Jalickee S, Miller SS. Retinal pigment epithelial function: a role for CFTR? *Doc Ophthalmol Adv Ophthalmol* 2003;106(1):43–50.

17. Constable PA, Lawrenson JG, Arden GB. Light and alcohol evoked electro-oculograms in cystic fibrosis. *Doc Ophthalmol Adv Ophthalmol* 2006;113(2):133–143.

18. Mulberg AE, Weyler RT, Altschuler SM, Hyde TM. Cystic fibrosis transmembrane conductance regulator expression in human hypothalamus. *Neuroreport* 1998;9(1):141–144.

19. Chalmers JA, Jang JJ, Belsham DD. Glucose sensing mechanisms in hypothalamic cell models: glucose inhibition of AgRP synthesis and secretion. *Mol Cell Endocrinol* 2014;382(1):262–270.

20. Weyler RT, Yurko-Mauro KA, Rubenstein R, et al. CFTR is functionally active in GnRH-expressing GT1-7 hypothalamic neurons. *Am J Physiol* 1999;277(3 Pt 1):C563-571.

21. Kim YS, Kim Y-B, Kim WB, et al. Histamine 1 receptor-Gβγ-cAMP/PKA-CFTR pathway mediates the histamine-induced resetting of the suprachiasmatic circadian clock. *Mol Brain* 2016;9(1):49.

22. Murray JM, Sletten TL, Magee M, et al. Prevalence of Circadian Misalignment and Its Association with Depressive Symptoms in Delayed Sleep Phase Disorder. *Sleep* 2016;e-Pub ahead of Print.

23. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag, Chronotype, and Cardiometabolic Risk. *J Clin Endocrinol Metab* 2015;100(12):4612–4620.

24. Carter SJ, Durrington HJ, Gibbs JE, et al. A matter of time: study of circadian clocks and their role in inflammation. *J Leukoc Biol* 2016;99(4):549–560.

25. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. *Nat Rev Cancer* 2003;3(5):350–361.

26. Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A* 2009;106(11):4453–4458.

27. Baron KG, Reid KJ. Circadian misalignment and health. *Int Rev Psychiatry Abingdon Engl* 2014;26(2):139–154.

28. Totterdell P, Reynolds S, Parkinson B, Briner RB. Associations of sleep with everyday mood, minor symptoms and social interaction experience. *Sleep* 1994;17(5):466–475.

29. Reid KJ, Abbott SM. Jet Lag and Shift Work Disorder. *Sleep Med Clin* 2015;10(4):523–535.

30. Asaoka S, Komada Y, Aritake S, Morita Y, Fukuda K, Inoue Y. Effect of delayed sleep phase during university life on the daytime functioning in work life after graduation. *Sleep Med* 2014;15(9):1155–1158.

31. Rajaratnam SMW, Howard ME, Grunstein RR. Sleep loss and circadian disruption in shift work: health burden and management. *Med J Aust* 2013;199(8):S11-15.

32. Kalmbach DA, Pillai V, Cheng P, Arnedt JT, Drake CL. Shift work disorder, depression, and anxiety in the transition to rotating shifts: the role of sleep reactivity. *Sleep Med* 2015;16(12):1532–1538.

33. Murray JM, Sletten TL, Magee M, et al. Abstract: Comparison of subjective sleep-wake characteristics and mood in Delayed Sleep Phase Disorder with and without an associated circadian phase angle disorder. *J Sleep Res* 2016;25(Suppl. 1):P352.

34. Nagtegaal JE, Laurant MW, Kerkhof GA, Smits MG, Meer YG van der, Coenen AM. Effects of melatonin on the quality of life in patients with delayed sleep phase syndrome. *J Psychosom Res* 2000;48(1):45–50.

35. Reid KJ, Zee PC. Circadian rhythm disorders. *Semin Neurol* 2004;24(3):315–325.

36. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152(3):1107–1136.

37. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005;26(2):319–338.

38. Morris AH, Kanner RE, Crapo RO, Gardner, Reed M. Clinical Pulmonary Function Testing: A Manual of Uniform Laboratory Procedures. 2nd ed. Intermountain Thoracic Soc; 1984.

39. Hankinson JL, John R. Odencrantz, Kathleen B. Fedan. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;159(1):179–187.

40. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40(6):1324–1343.

41. Liou TG, Adler FR, Keogh RH, et al. Sputum biomarkers and the prediction of clinical outcomes in patients with cystic fibrosis. *PloS One* 2012;7(8):e42748.

42. Roenneberg T, Wirz-Justice A, Merrow M. Life between Clocks: Daily Temporal Patterns of Human Chronotypes. *J Biol Rhythms* 2003;18(1):80–90.

43. Zavada A, Gordijn MCM, Beersma DGM, Daan S, Roenneberg T. Comparison of the Munich Chronotype Questionnaire with the Horne-Ostberg's Morningness-Eveningness Score. *Chronobiol Int* 2005;22(2):267–278.

44. Terman JS, Terman M, Lo ES, Cooper TB. Circadian time of morning light administration and therapeutic response in winter depression. *Arch Gen Psychiatry* 2001;58(1):69–75.

45. Zar JH. Biostatistical Analysis. 4th ed. Prentice Hall; 1998.

46. R Development Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2010. Available from: http://www.R-project.org

47. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian typology: a comprehensive review. *Chronobiol Int* 2012;29(9):1153–1175.

48. Bahammam AS, Alaseem AM, Alzakri AA, Almeneessier AS, Sharif MM. The relationship between sleep and wake habits and academic performance in medical students: a cross-sectional study. *BMC Med Educ* 2012;12:61.

49. Curcio G, Ferrara M, De Gennaro L. Sleep loss, learning capacity and academic performance. *Sleep Med Rev* 2006;10(5):323–337.

50. Yazdi Z, Sadeghniiat-Haghighi K, Loukzadeh Z, Elmizadeh K, Abbasi M. Prevalence of Sleep Disorders and Their Impacts on Occupational Performance: A Comparison between Shift Workers and Nonshift Workers. *Sleep Disord* 2014;2014:870320.

51. Figueiro MG, White RD. Health consequences of shift work and implications for structural design. *J Perinatol Off J Calif Perinat Assoc* 2013;33 Suppl 1:S17-23.

52. Rosenfeld M, Emerson J, Williams-Warren J, et al. Defining a pulmonary exacerbation in cystic fibrosis. *J Pediatr* 2001;139(3):359–365.

53. Sawicki GS, Ayyagari R, Zhang J, et al. A pulmonary exacerbation risk score among cystic fibrosis patients not receiving recommended care. *Pediatr Pulmonol* 2013;48(10):954–961.

54. Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of Aerosolized Recombinant Human DNase on Exacerbations of Respiratory Symptoms and on Pulmonary Function in Patients with Cystic Fibrosis. *N Engl J Med* 1994;331(10):637–642.

55. Chang A-M, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. *Proc Natl Acad Sci U S A* 2015;112(4):1232–1237.

56. Lewy AJ, Cutler NL, Sack RL. The endogenous melatonin profile as a marker for circadian phase position. *J Biol Rhythms* 1999;14(3):227–236.

57. Molina TA, Burgess HJ. Calculating the dim light melatonin onset: the impact of threshold and sampling rate. *Chronobiol Int* 2011;28(8):714–718.

58. Jankelowitz L, Reid KJ, Wolfe L, Cullina J, Zee PC, Jain M. Cystic fibrosis patients have poor sleep quality despite normal sleep latency and efficiency. *Chest* 2005;127(5):1593–1599.

Figure Legends

Figure 1. Sleep Phase Measurements and Clinical Data for Patients with CF

A. We found no relationship within patients with CF between lung function, prior acute pulmonary exacerbations or weight with sleep latency or total sleep duration. Compared to controls, patients with CF had longer sleep latency that was significant without adjustment and prolonged sleep duration that was borderline in significance (Table 2A). However, both latency and duration were significantly longer in patients with CF after adjustment for age, sex and height (Table 2B).

B. Timing of sleep onset, mid-sleep and wake-up times were independent of FEV_1%, 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 2A), and the associations were strengthened after adjustments for age, sex and height as appropriate (Table 2B, e-Table 1A-C).

Figure 2. Relationship between Prior Acute Pulmonary Exacerbations and FEV_1%

FEV_1% normalized using NHANES III equations and the number of acute pulmonary exacerbations in the prior year (truncated to a maximum of 5) are the two most important predictors of survival in CF.[7] Patients with CF that participated in this study had a wide range of FEV_1% values and number of prior acute pulmonary exacerbations requiring hospitalization. Using quasi-Poisson regression, we detected a strong inverse relationship. Note that acute pulmonary exacerbations counts were jittered to better show individual values.

Table 1] Demographics of Study Participants

Characteristic*	Patients with CF, N = 45	Control Volunteers without CF, N = 48	*P* value
Number of Females (Percent)	23 (51)	26 (54)	NS
Age	29.6 (6.8, 19.0-47.0)	27.4 (6.7, 18.0-45.0)	0.007
Height, cm	168.1 (9.5, 152.0-187.4)	174.3 (9.0, 158.0-190.0)	0.002
Weight, kg	62.3 (14.5, 37.9-98.0)	--	--
FEV_1%, Percent Predicted (NHANES III)	62.0 (23.8, 19.0-105.0)	--	--
FEV_1%, Percent Predicted (GLI)	62.1 (23.8, 18.9-104.2)	–	--
Number of Prior acute pulmonary exacerbations	2.2 (2.7, 0-14)	--	--
Number F508del Homozygotes (Percent)	24 (53)	--	--

*Data are presented as mean values (SD, Range) except as noted.

Table 2A] Sleep Phase Delay due to CF, Univariate Models

Sleep Measure	Estimate*	SE*	*P*
Sleep-onset Time	0.547	0.263	0.04
Mid-sleep Time	0.781	0.243	0.002
Wake-up Time	1.01	0.288	< 0.001
Sleep Latency (Minutes)	8.610	3.670	0.02
Sleep Duration	0.467	0.259	0.07

*Sleep measure estimates and SE are in hours except as noted.

Table 2B] Sleep Phase Delay due to CF, Multivariable Models adjusted by Age, Sex and Height*

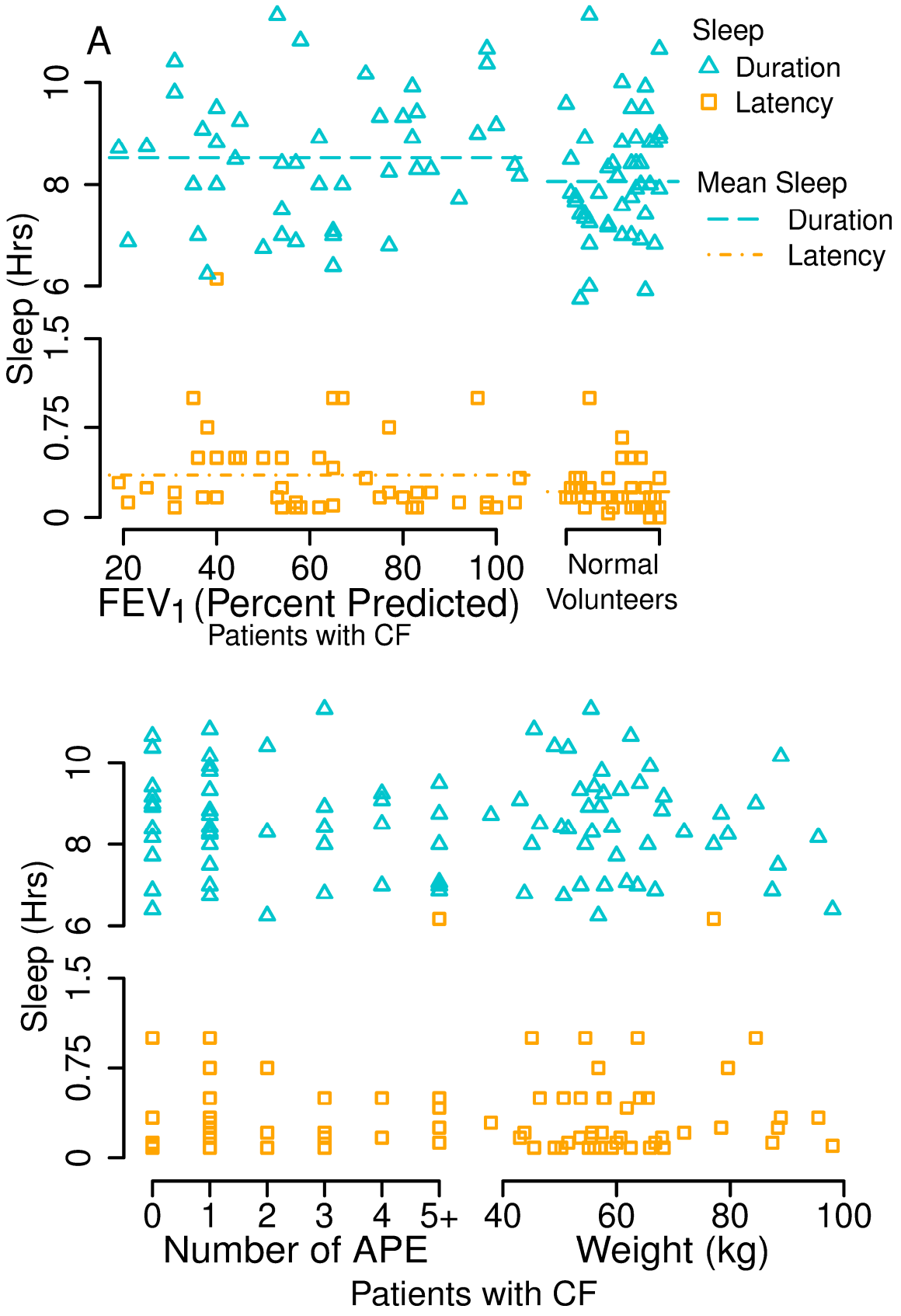
Sleep Measure	Estimate*	SE*	*P*
Sleep-onset Time	0.612	0.247	0.015
Mid-sleep Time	1.110	0.246	< 0.001
Wake-up Time	1.150	0.279	< 0.001
Sleep Latency (Minutes)	7.210	3.630	0.05
Sleep Duration	0.489	0.250	0.05

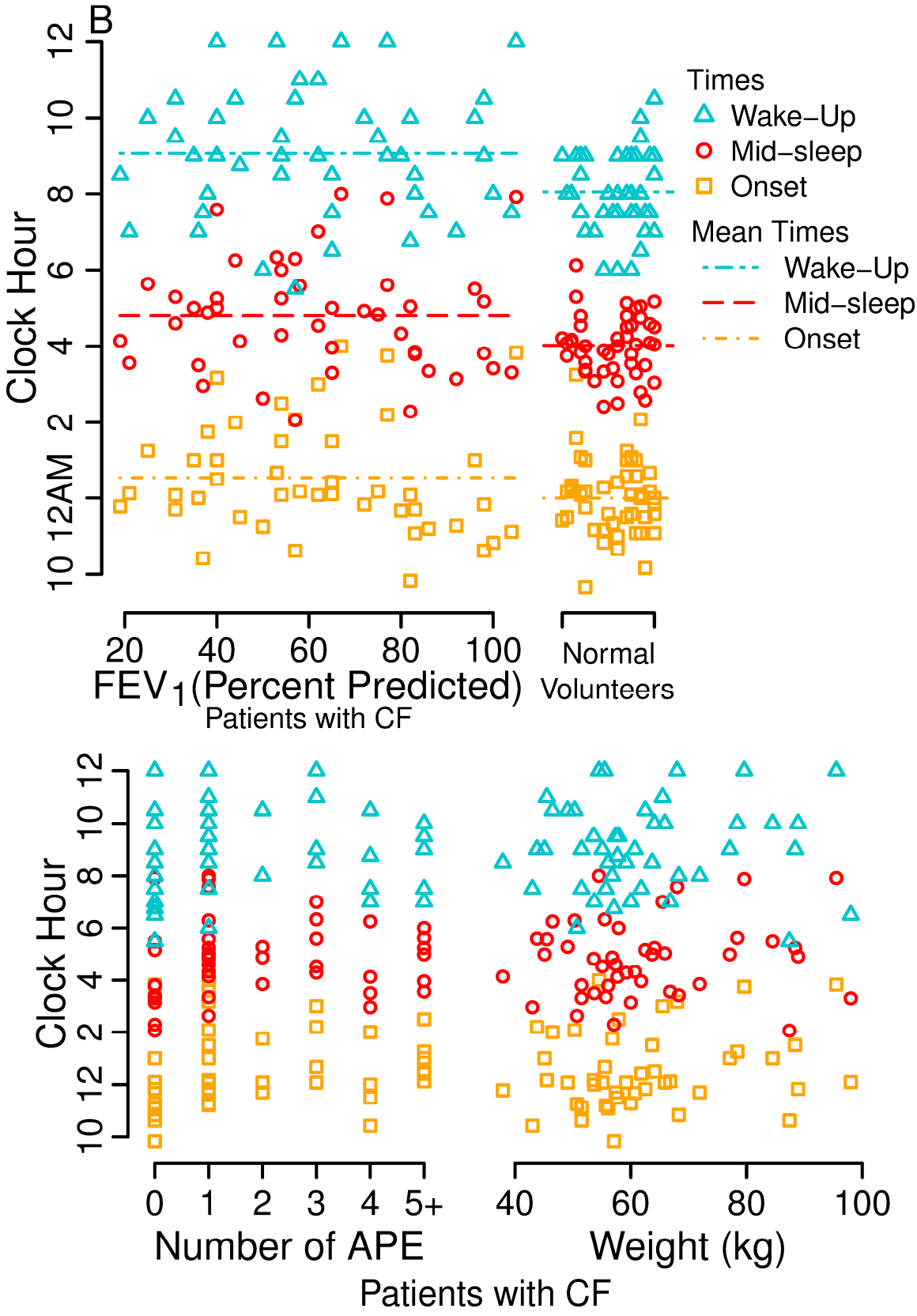
*Each estimate is the effect of CF as an explanatory variable adjusted for age, sex and height when statistically significant. The estimates of effects for statistically significant adjustment coefficients are shown in e-Table 1. Estimates and SE are in hours except as noted.

Table 2C] Sleep Phase Delay Associated with Later Sleep-onset Times in CF

Participants	Sleep Outcome Affected by Later Sleep-onset times	Estimate*	SE	P
Patients with CF	Sleep Latency (Min)	7.510	1.920	< 0.001
	Mid-sleep Time	0.854	0.0584	< 0.001
	Wake-up Time	0.708	0.117	< 0.001
	Sleep Duration	-0.292	0.117	0.016
Volunteers without CF	Mid-sleep Time	0.625	0.0741	< 0.001
	Sleep Duration	-0.750	0.148	< 0.001

*Estimates 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 each non-significant.


A
Sleep (Hrs)
0
0.75
1.5
6
8
10
Sleep
Duration
Latency
Mean Sleep
Duration
Latency
20
40
60
80
100
FEV1 (Percent Predicted)
Patients with CF
Normal
Volunteers
0
1
2
3
4
5+
Number of APE
40
60
80
100
Weight (kg)
Patients with CF


B
Clock Hour
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Times
Wake–Up
Mid–sleep
Onset
Mean Times
Wake–Up
Mid–sleep
Onset
20 40 60 80 100
FEV1 (Percent Predicted)
Patients with CF
Normal Volunteers
Clock Hour
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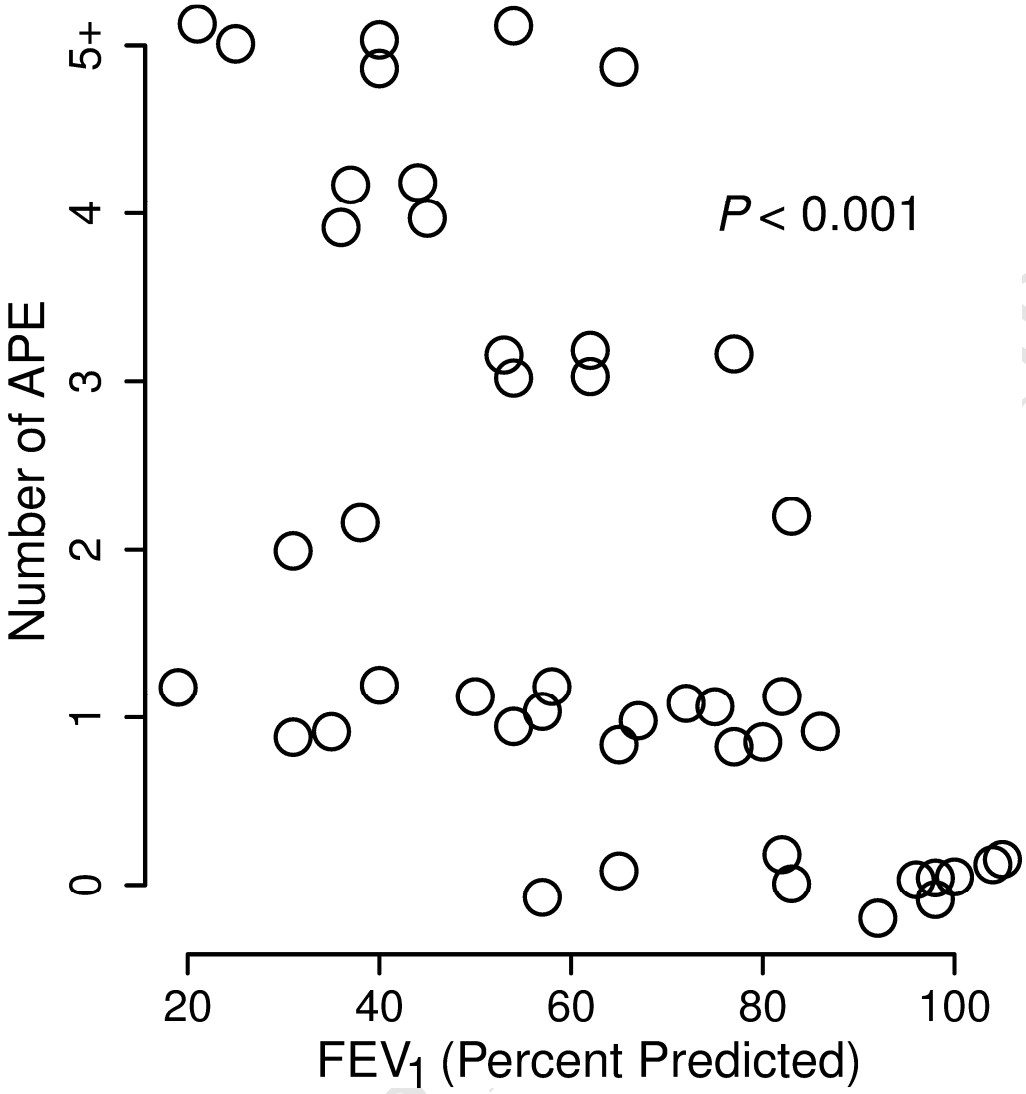
0 1 2 3 4 5+
Number of APE
40 60 80 100
Weight (kg)
Patients with CF



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e-Table 1A] Multivariable Model of Sleep-onset Time

	Estimate*	SE*	P	95% CI*
Intercept	0.731	0.529	0.17	-0.306, 1.77
Group (CF=1)	0.612	0.247	0.015	0.128, 1.10
Age (Yrs)	-0.0419	0.0183	0.024	-0.078, -0.006
Sex (Male=0)	0.886	0.245	< 0.001	0.406, 1.37
*Estimates, SE and 95% CI are in hours.				

e-Table 1B] Multivariable Model of Mid-sleep Time

	Estimate*	SE*	P	95% CI*
Intercept	-0.122	2.18	0.955	-4.39, 4.15
Group (CF=1)	1.11	0.246	<0.001	0.628, 1.59
Age (Yrs)	-0.056	0.017	0.001	-0.090, -0.023
Height (cm)	0.033	0.018	0.01	0.008, 0.058
*Estimates, SE and 95% CI are in hours.				

e-Table 1C] Multivariable Model of Wake-up Time

	Estimate*	SE*	P	95% CI*
Intercept	9.74	0.594	<0.001	8.58, 10.9
Group (CF=1)	1.15	0.279	<0.001	0.603, 1.70
Age (Yrs)	-0.062	0.021	0.003	-0.102, -0.021
*Estimates, SE and 95% CI are in hours.				

e-Table 1D] Multivariable Model of Sleep Latency

	Estimate*	SE*	P	95% CI*
Intercept	-4.65	7.72	0.548	-19.8, 10.5
Group (CF=1)	7.21	3.63	0.05	0.095, 14.3
Age (Yrs)	0.64	0.267	0.02	0.117, 1.16
*Estimates, SE and 95% CI are in minutes.				

e-Table 1E] Multivariable Model of Sleep Duration

	Estimate*	SE*	P	95% CI*
Intercept	8.38	0.209	<0.0019	7.97, 8.79
Group (CF=1)	0.489	0.250	0.05	0, 0.979
Sex (Male=0)	-0.703	0.251	0.01	-1.19, -0.21
*Estimates, SE and 95% CI are in hours.				

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e-Table 2A] Effects on Sleep Latency of Later Sleep-onset Times Adjusted by Age in CF

	Estimate	SE	P	95% CI
Intercept	-23.9	13.1	0.0744	-49.6, 1.78
Sleep-onset Time (Hrs)	7.51	1.92	0.000331	3.75, 11.3
Age (Yrs)	1.4	0.424	0.00196	0.569, 2.23

*Estimates, SE and 95% CI are in hours.

e-Table 2B] Effects on Mid-sleep Time of Later Sleep-onset Times Adjusted by Age in CF

	Estimate	SE*	*P**	95% CI
Intercept	5.41	0.398	<0.001	4.63, 6.19
Sleep-onset Time (Hrs)	0.854	0.0584	<0.001	0.740, 0.968
Age (Yrs)	-0.0361	0.0129	0.008	-0.061, -0.011

*Estimates, SE and 95% CI are in hours.

e-Table 2C] Effects on Wake-up Time of Later Sleep-onset Times Adjusted by Age in CF

	Estimate	SE*	*P**	95% CI
Intercept	10.8	0.796	<0.001	9.24, 12.4
Sleep-onset Time (Hrs)	0.708	0.117	<0.001	0.479, 0.937
Age (Yrs)	-0.0724	0.0258	0.008	-0.123, -0.022

*Estimates, SE and 95% CI are in hours.

e-Table 2D] Effects on Sleep Duration of Later Sleep-onset Times Adjusted by Age in CF

	Estimate	SE*	*P**	95% CI
Intercept	10.8	0.796	<0.001	9.24, 12.4
Sleep-onset Time (Hrs)	-0.292	0.117	0.02	-0.521, -0.0627
Age (Yrs)	-0.0724	0.0258	0.008	-0.123, -0.022

*Estimates, SE and 95% CI are in hours.

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e-Table 3] Linear Regression of Global Lung Initiative and NHANES III FEV_1% Values

	Estimate	SE	*P*
Intercept	0.114	0.504	0.82
FEV_1% (NHANES III)	0.998	0.008	<0.001
Multiple R^2 = 0.998; Adjusted R^2 = 0.997.			

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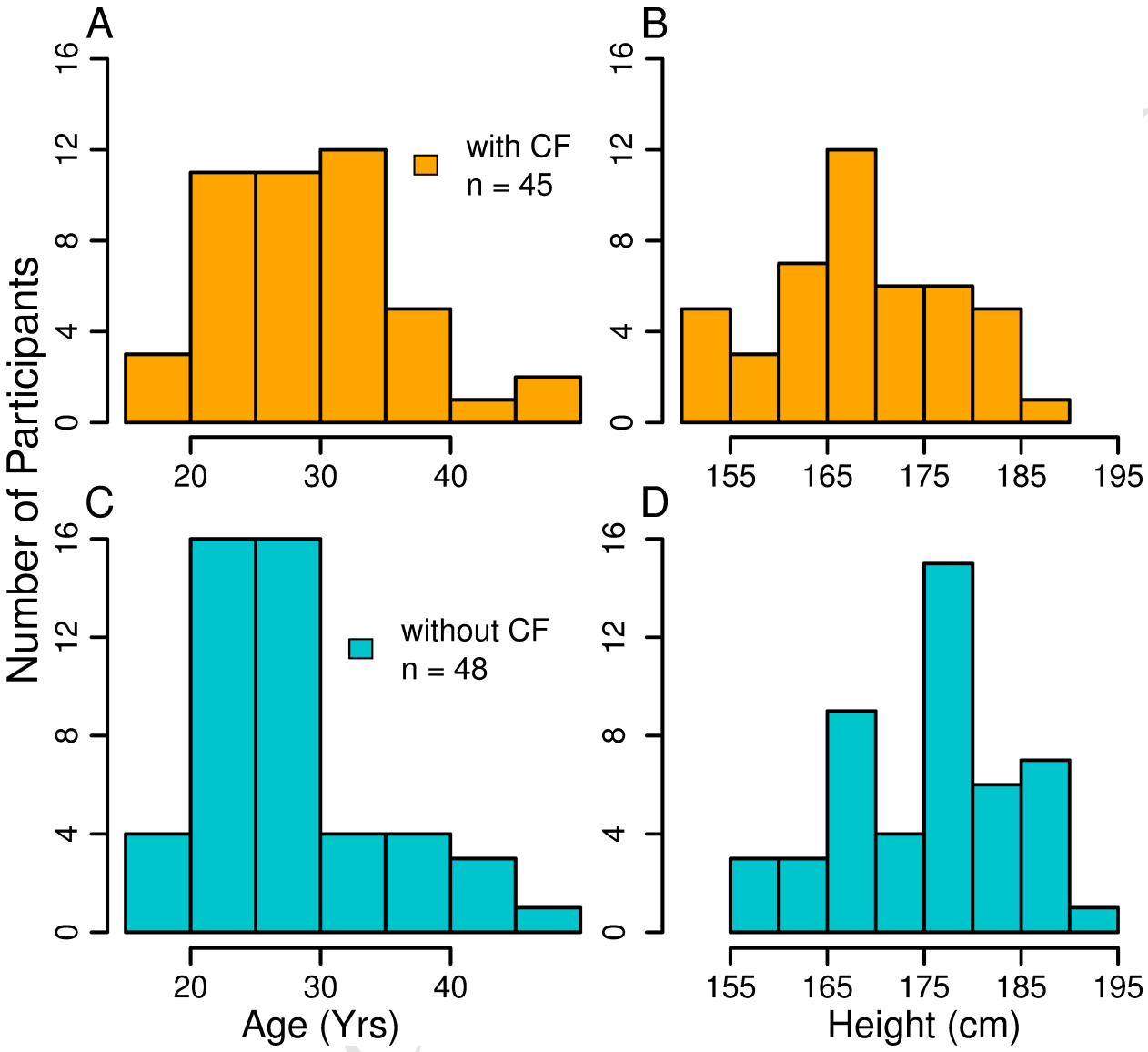
e-Figure 1. Histograms of Age and Height of Study Participants



Comparisons of **A. Age** and **B. Height in Patients with CF** with **C. Age** and **D. Height in Volunteers without CF**, respectively, show that patients with CF were slightly older (Kolmogorov-Smirnov test, $P = 0.007$) and slightly shorter than volunteers without CF (Kolmogorov-Smirnov test, $P = 0.002$). These differences require adjustment of models of sleep phase measurements because of the statistically significant relationships between sleep phase , age, sex and height (Table 2B and e-Table-1).[35]

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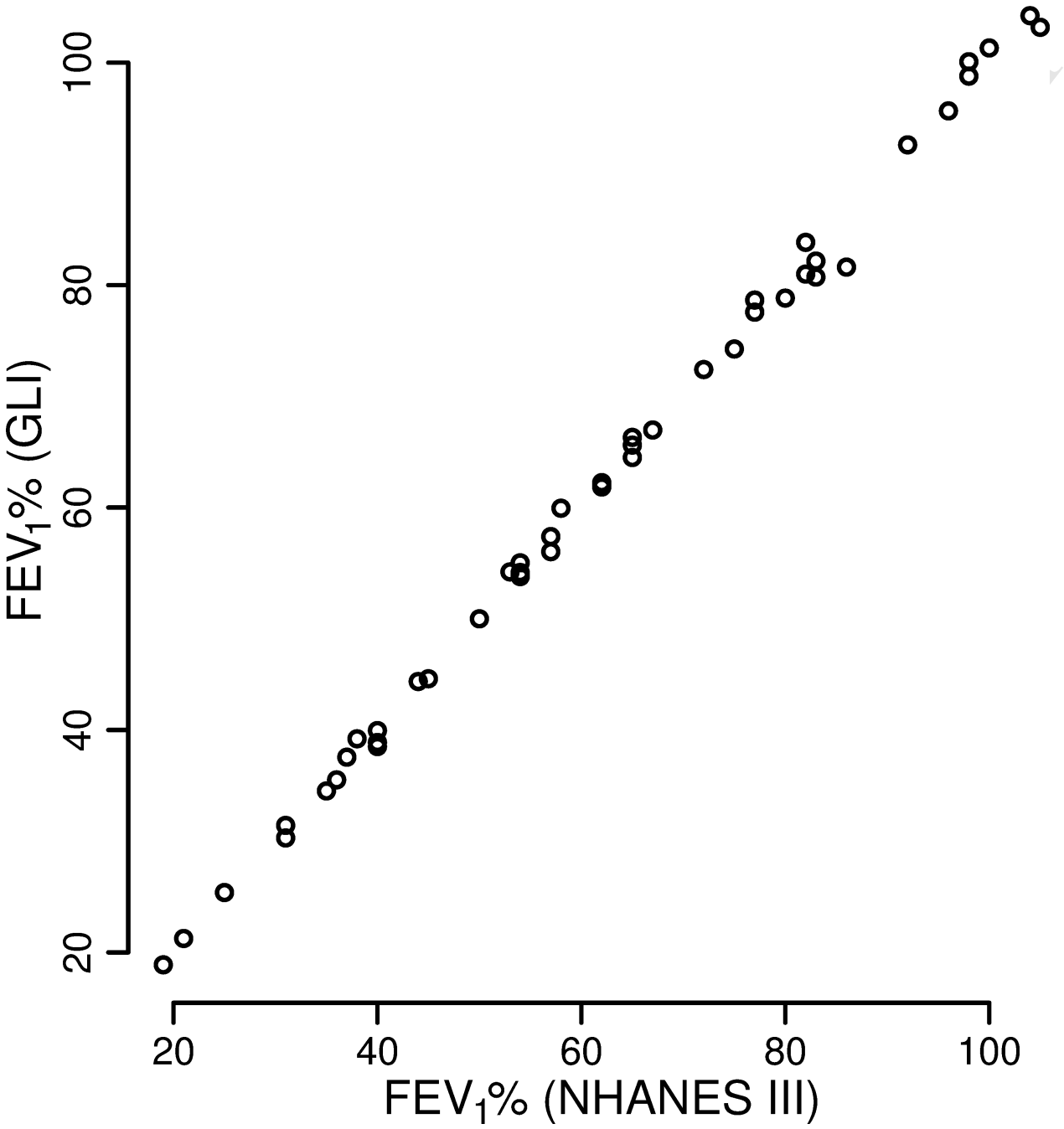
e-Figure 2. Comparison of FEV_1% normalization by NHANES III and GLI equations.



After measuring FEV_1, we used NHANES III equations to normalize values for age, sex, height, race and ethnicity; we used GLI equations to normalize the same values for age, sex, height and race. The display shows extremely small differences between the two sets of normalizations. See also e-Table 3.

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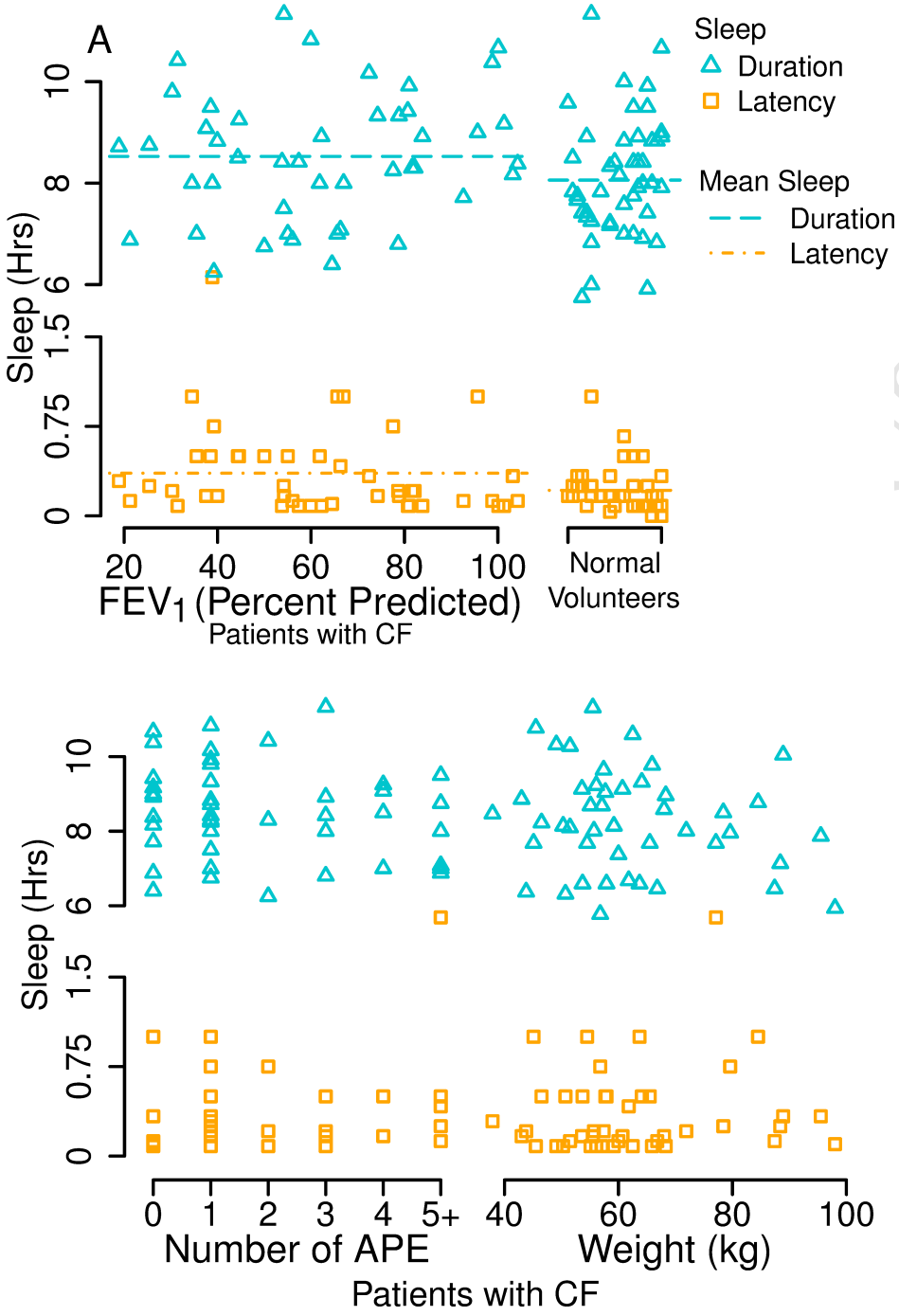
e-Figure 3. Sleep Phase Measurements and Clinical Data for Patients with CF using FEV1% normalized using GLI equations.



A. Results are the same as shown in Figure 1A when GLI equations are used instead of NHANES III equations to normalize FEV_1%. As in Figure 1A, we found no relationship within patients with CF between lung function, prior acute pulmonary exacerbations or weight with sleep latency or total sleep duration. Compared to controls, patients with CF had longer sleep latency that was significant without adjustment and prolonged sleep duration that was borderline in significance (Table 2A). However, both latency and duration were significantly longer in patients with CF after adjustment for age, sex and height (Table 2B).

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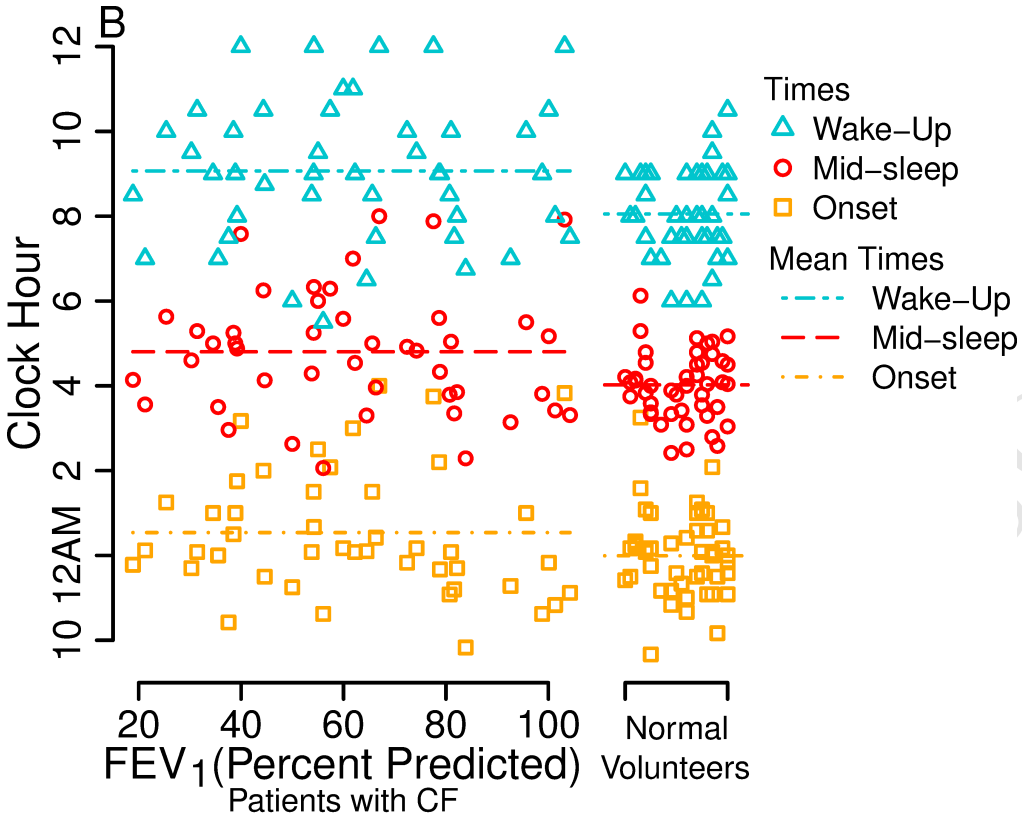

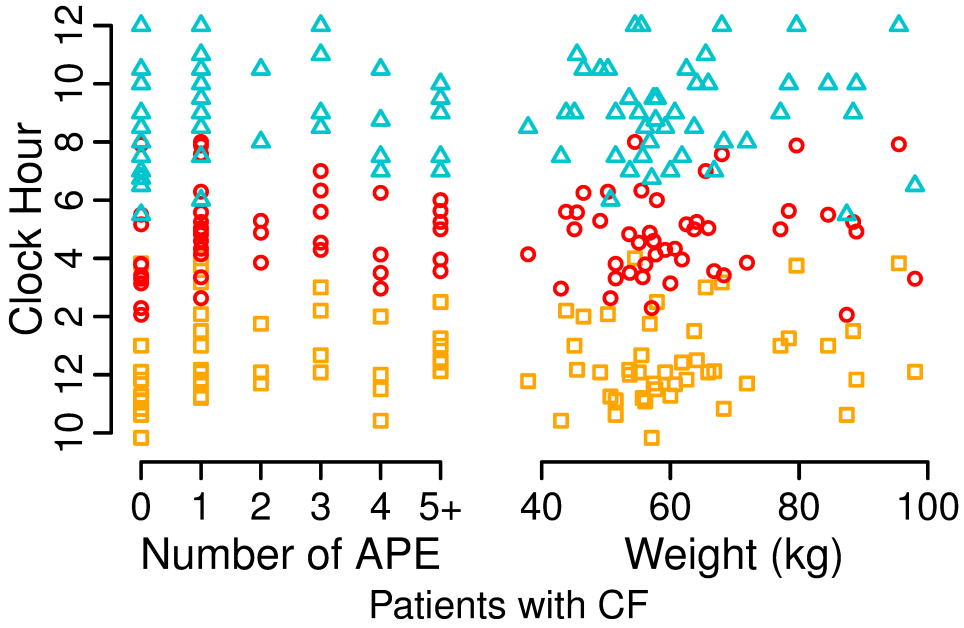

B. Results are the same as shown in Figure 1B when GLI equations are used instead of NHANES III equations to normalize FEV_1%. As in Figure 1B, timing of sleep onset, mid-sleep and wake-up times were independent of FEV_1%, number of acute pulmonary exacerbations in the prior year and weight among patients with CF. Volunteers without CF had earlier times for all three sleep events. The differences were significant without adjustment, and the associations were strengthened after adjustments for age, sex and height as appropriate.

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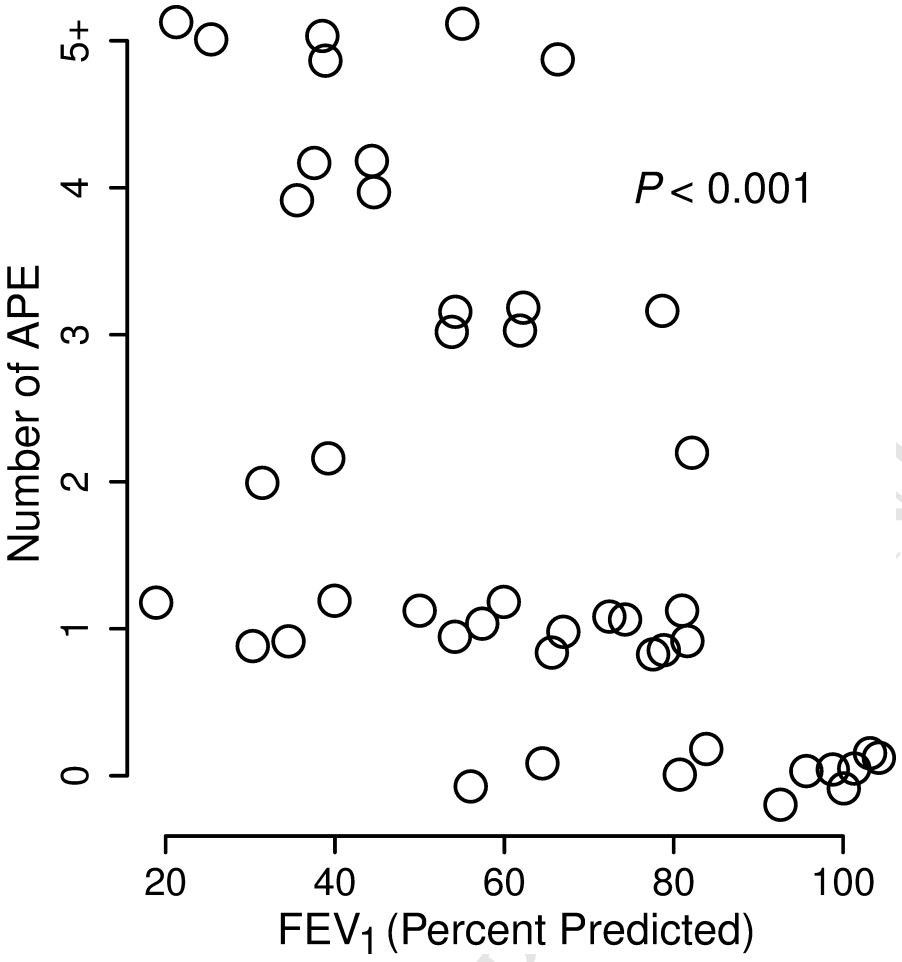
e-Figure 4. Relationship between Prior Acute Pulmonary Exacerbations and FEV_1% normalized using GLI equations.



FEV_1% and the number of acute pulmonary exacerbations in the prior year (truncated to a maximum of 5) are the two most important predictors of survival in CF.[7] Patients with CF that participated in this study had a wide range of FEV_1% values and number of prior acute pulmonary exacerbations. Using quasi-Poisson regression, we detected a strong inverse relationship. Note that acute pulmonary exacerbations counts have been jittered to better show individual values.