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5 **A novel spirometric measure identifies mild chronic obstructive pulmonary disease**
6 **unidentified by standard criteria**

7 **Short Title:** A novel spirometric criterion for mild COPD

8

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20

21 This article has an online data supplement.

1 **Abstract**

2 **Rationale:** In chronic obstructive pulmonary disease both smaller and larger airways are
3 affected. Forced expiratory volume in one second (FEV_1) mainly reflects large airways
4 obstruction, while the later fraction of forced exhalation reflects reduction in terminal expiratory
5 flow.

6 **Objective:** To evaluate the relationship between spirometric ratios, including the ratio of forced
7 expiratory volume in 3 and 6 seconds (FEV_3/FEV_6), and small airway measures and gas trapping
8 in quantitative chest computed tomography (CT), and clinical outcomes in the COPDGene
9 cohort.

10 **Methods:** 7,853 current and ex-smokers were evaluated for airflow obstruction using recently-
11 defined linear iteratively-derived equations of Hansen et al.¹ to determine lower limits of normal
12 equations for pre-bronchodilator FEV_1/FVC , FEV_1/FEV_6 , FEV_3/FEV_6 and FEV_3/FVC . General
13 linear and ordinal regression models were applied to the relation between pre-bronchodilator
14 spirometry and radiologic and clinical data.

15 **Main Results:** Of the 10,311 participants included in the COPDGene Phase 1 study, participants
16 with incomplete quantitative CT or relevant spirometric data were excluded, resulting in 7,853
17 participants in the present study. Of 4,386 participants with ratio of FEV_1 to forced vital capacity
18 (FEV_1/FVC) greater than lower limit of normal, 15.4% had abnormal FEV_3/FEV_6 . Compared to
19 participants with normal FEV_3/FEV_6 and FEV_1/FVC , abnormal FEV_3/FEV_6 was associated with
20 significantly greater gas trapping, St. George Respiratory Questionnaire score, mMRC dyspnea
21 score, BODE index, and shorter six-minute walking distance (all $P < 0.0001$), but not CT-
22 evidence of emphysema.

1 **Conclusions:** Current and ex-smokers with pre-bronchodilator $FEV_3/FEV_6 <$ lower limit of
2 normal as the sole abnormality identifies a distinct population with evidence of small airway
3 disease in quantitative CT, impaired indices of physical function and quality of life otherwise
4 deemed normal by current spirometric definition.

5 **Key words:** airway obstruction, spirometry, thoracic radiology, COPD

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1 **Introduction**

2 Expiratory airflow obstruction is the key finding supporting chronic obstructive
3 pulmonary disease (COPD) diagnosis. Airflow obstruction prevalence varies widely by
4 definition used¹⁻⁵. Recently, Hansen et al. defined equations characterizing pre-bronchodilator
5 normal values for several spirometric variables using the Third National Health and Nutrition
6 Examination Survey (NHANES) database¹. For this analysis, lower limits of normal (LLN)
7 resides at 5th percentile of each decade of age, and results in a more balanced estimation of LLN
8 than previous approaches^{6,7}. These new reference ranges were further evaluated here¹.

9 Small airways are frequently involved early in the course of COPD with significant
10 pathologic changes before symptom onset and spirometry changes⁸⁻¹⁰. Forced expiratory volume
11 in one second (FEV₁) mainly reflects large airways obstruction. Since the later fraction of forced
12 exhalation (e.g. FEV₃) better reflects smaller airway contributions, it may be a more sensitive
13 measure to diagnose early airway obstruction in COPD^{11,12}. Spirometric ratios have less
14 variability than timed forced expirations^{12,13}. Accumulating evidence suggests many current or
15 former smokers have clinical, radiological and physiological abnormality not identified by
16 currently-used spirometric measures^{9,14-16}. The FEV₁ forced vital capacity (FVC) ratio may
17 remain within the “normal” range even after considerable airway damage has occurred.
18 FEV₃/FEV₆ and FEV₃/FVC have been proposed as measures capable of detecting small airways
19 disease hence mild COPD manifestations^{11,17}. Supportingly, a greater number of smokers were
20 found below LLN for FEV₃/FEV₆ than for FEV₁/FEV₆¹ and below LLN for FEV₃/FVC than for
21 FEV₁/FVC^{1,18}. However, the clinical importance of an isolated low pre-bronchodilator
22 FEV₃/FEV₆ or FEV₃/FVC in smokers remains unknown.

1 We aimed to determine whether $FEV_3/FEV_6 < LLN$ and $FEV_3/FVC < LLN$ were associated
2 with quantitative computed tomography (CT) and other COPD-related clinical outcomes in
3 subjects with normal pre-bronchodilator spirometry by standard criteria (e.g., $FEV_1/FVC > LLN$)
4 in the COPDGene cohort¹⁹. Quantitative computed tomography (CT) is useful for *in vivo*
5 assessment of lung morphological changes and provides visual and quantitative assessment of
6 COPD^{20,21}. In particular, quantitative CT is useful to quantify emphysema percentage and
7 distribution, airway dimensions changes, and gas trapping severity in COPD²²⁻²⁴. We
8 hypothesized that abnormal FEV_3/FEV_6 and FEV_3/FVC would be associated with quantitative
9 CT abnormalities and adverse clinical manifestations.

10

11 **Materials and Methods**

12 *Study population and data collection*

13 We utilized data from COPDGene for participants enrolled between 2007 and 2011¹⁹.
14 The cohort includes 10,311 non-Hispanic white and African-American men and women, 45-80
15 years old, with ≥ 10 pack-years smoking history. COPDGene study excluded those with
16 pregnancy, previous lung resection surgery, active cancer treatment, or history of lung disease
17 other than asthma and COPD¹⁹. For the present study, excluding those with incomplete CT and
18 spirometric data yielded 7,853 participants (see *online supplement*). As clinical and functional
19 correlates, we used St. George's Respiratory Questionnaire (SGRQ)²⁵ (permission was obtained
20 for use of this instrument), modified Medical Research Council (mMRC) dyspnea scale²⁶ and
21 BODE (body mass index, airflow obstruction, dyspnea, exercise capacity) scores²⁷, and six-
22 minute walking distance (6MWD)²⁸. Institutional Review Boards approved the study at 21
23 participating centers (see *online supplement*). Participants provided written informed consent.

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Spirometry and quality control

Spirometry was performed using an ultrasound-based spirometer (EasyOne™, Model 2001, NDD Medizintechnik AG, Zurich, Switzerland) before and after albuterol administration following ERS/ATS recommendations²⁹. Positive bronchodilator response was defined as an increase in FEV₁ and/or FVC \geq 12% of baseline and 200 ml²⁹. *Online supplement* presents quality control assurance details.

In our analysis, we defined LLN criteria abnormality originating from spirometry data of the Third NHANES, in which only pre-bronchodilator responses were collected¹. For our analysis, values of the pre-bronchodilator FEV₁, FVC, FEV₃, and FEV₆ from the best test were chosen; best test was defined as the maneuver with the largest FVC and FEV₁ sum. % predicted values were calculated from NHANES-III equations^{6,30}.

Quantitative CT analysis

CT scans were acquired at full inspiration and after tidal expiration (see *online supplement*). Emphysema is defined as morphologically loss of alveolar tissue³¹. Percentage of low attenuation areas below -950 Hounsfield Units (HU) on end-inspiratory CT scan is thought to represent emphysema²². Airway disease was assessed by segmental airway wall area percentage³². Two additional measurements assessed small airway disease by means of expiratory scans. Gas trapping% was defined as %lung voxels below -856 HU on expiratory scans³³. Expiratory/inspiratory ratio of mean lung attenuation (E/I MLA) was defined as the ratio of mean lung attenuation from density histograms on inspiratory and expiratory scans³⁴.

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Statistical analyses

SPSS 22.0 procedures were used (Armonk, NY: IBM Corp.) (also see *online supplement*). Relationships between spirometry (independent variable) and CT, clinical and functional correlates (dependent variables) were assessed by multiple linear regression models using age, sex, race, pack-year smoking, body mass index (BMI) and CT scanner type (only for CT measures) as covariates. Covariates were retained if $P < 0.10$. Quantitative CT, SGRQ and 6MWD were log transformed; regression coefficients (β) were then back-transformed to aid interpretation. Compared to a reference category, relative differences for outcome variables were determined by $(e^{\beta} - 1) \times 100\%$, while holding other predictors constant (see *online supplement*). A proportional odds model was used for ordinal outcomes (BODE and mMRC). Analyses were performed separately for the whole study population and the $FEV_1/FVC \geq LLN$ subgroup. $P < 0.05$ was considered statistically significant.

To define impairments in the $FEV_1/FVC \geq LLN$ subgroup, we selected explanatory variables predicting important outcomes and reasonable thresholds in COPD: FEV_1 %predicted $< 65\%$, mMRC scale dyspnea ≥ 2 , 6MWD < 350 meters, SGRQ > 25 and BODE score $> 2^{27,35}$. To define clinically meaningful radiologic abnormality, we used newly described cutoffs: 3.5 for emphysema% and 21 for gas trapping%³⁶.

Results

Spirometric characterization

Subject characteristics for the 7,853 participants are presented in Table 1. Pre-bronchodilator FEV_1/FVC was $< LLN$ in 3,467 (44.1%) participants, thus defining, by standard

1 criteria, those having airflow obstruction; the remaining 4,386 (55.9%) had no recognizable
2 airflow obstruction.

3 4 *Abnormality of FEV₁/FVC and FEV₃/FEV₆ in the overall group*

5 In the overall group, participants <LLN for either FEV₁/FVC (3,467; 44.1%) or
6 FEV₃/FEV₆ (3,965; 50.5%) criteria were older, had lower BMI, longer smoking history and were
7 more likely Caucasian. These individuals also had lower spirometric measurements, higher
8 pulmonary structural impairments, dyspnea, SGRQ and BODE scores and shorter 6MWD (Table
9 1). Correlates of FEV₁/FVC and FEV₃/FEV₆ abnormality in the overall subject group are
10 presented in *online supplement*, e-Table 1.

11 12 *Correlates of FEV₃/FEV₆ abnormality in participants with FEV₁/FVC ≥LLN*

13 Of subjects with normal FEV₁/FVC, more (677, 15.4%) had FEV₃/FEV₆ <LLN than
14 FEV₃/FVC <LLN (312, 7.1%). Those with FEV₃/FEV₆ <LLN had significantly worse
15 spirometric, clinical outcomes and CT indices (with exception of emphysema %) than those with
16 FEV₃/FEV₆ ≥LLN (Figure 1, Table 2). While individuals with FEV₃/FVC <LLN had
17 significantly worse spirometry and SGRQ, their quantitative CT results (except E/I MLA) were
18 not significantly different from those with FEV₃/FVC ≥LLN (Table 2).

19 Importantly, association of FEV₃/FEV₆ abnormality with structural and clinical outcomes
20 was significant –though somewhat weaker than in the overall group (e-Table 1)– in non-
21 obstructed participants (FEV₁/FVC ≥LLN) (Table 3): gas trapping% was 27.5% greater, WA%
22 was 1.8% greater and E/I MLA was 2.0% greater than those with FEV₃/FEV₆ ≥LLN. We also
23 observed 40.8% greater SGRQ and 7.2% shorter 6MWD in those with abnormal FEV₃/FEV₆.

1 They were also more likely to be in higher mMRC category (adjusted OR: 1.6, 95% CI: 1.4 –
2 1.9, P<0.0001) and BODE index (adjusted OR: 2.8, 95% CI: 2.1 – 3.7, P<0.0001) compared
3 with $FEV_3/FEV_6 \geq LLN$ subjects (Table 3).

4 Finally, to better understand if reversible smooth muscle contraction was a significant
5 determinant of these findings, we excluded 1,708 (21.7%) participants with a positive
6 bronchodilator response. Results in those 3,868 with negative bronchodilator response and with
7 $FEV_1/FVC \geq LLN$ showed essentially the same associations of FEV_3/FEV_6 abnormality as in the
8 wider group (e-Table 2).

9 Multivariable analyses in the subgroup without airflow obstruction but with FEV_3/FVC
10 $< LLN$ (Table 4) showed significant associations with CT parameters reflecting small airways
11 disease (gas trapping% and E/I MLA), SGRQ, mMRC and BODE. Notably, however, strength of
12 these associations was lower than for FEV_3/FEV_6 (Tables 3 and 4).

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14 *Impairments in subjects with FEV_3/FEV_6 abnormality and $FEV_1/FVC \geq LLN$*

15 Among those with normal spirometry by FEV_1/FVC criteria, impairments in radiological
16 and clinical outcomes were significantly more common among those with $FEV_3/FEV_6 < LLN$
17 compared to $FEV_3/FEV_6 \geq LLN$ participants, except CT evidence of emphysema (Figure 2, e-
18 Table 5). This finding further supports that abnormal FEV_3/FEV_6 in patients with otherwise
19 normal spirometry have worse clinical symptoms, less functional capacity and worse air
20 trapping, but not significantly greater emphysema%.

21

22 **Discussion**

1 We identified 677 (15.4%) current and former smokers classified as non-obstructed using
2 standard pre-bronchodilator spirometric criteria ($FEV_1/FVC \geq LLN$) in the COPDGene
3 population in whom FEV_3/FEV_6 was abnormal. This group with FEV_3/FEV_6 as the sole
4 abnormality showed impairments in quantitative CT indices (gas trapping%, E/I MLA and
5 segmental wall area, but not emphysema%) as well as shorter 6MWD, increased mMRC, SGRQ
6 and increased BODE scores. We believe that this analysis establishes a criterion that detects
7 mild structural, functional and clinical abnormalities in subjects otherwise deemed normal by
8 standard spirometric definitions.

9 Increasing evidence suggests that a large fraction of current or former smokers have
10 clinical, radiological and physiological disease not consistently identified by spirometry^{9,14-16}.
11 FEV_3/FEV_6 and FEV_3/FVC identified significantly more subjects below LLN than FEV_1/FVC
12 and FEV_1/FEV_6 in the never-smoker NHANES-III population, but the clinical importance of
13 these abnormalities was unknown¹.

14 Our study is the first to extensively characterize smokers with FEV_3/FVC or FEV_3/FEV_6
15 as the sole abnormality with respect to pulmonary structural impairment, functional and patient-
16 reported outcomes. We found abnormality in small airway measures (gas trapping% and E/I
17 MLA), segmental airway wall area, $FEV_1\%pred$, $FEV_1/FVC \%pred$, SGRQ, mMRC, BODE and
18 6MWD each associated with abnormal FEV_3/FEV_6 (Tables 1-3).

19 Compared with FEV_1/FVC , FEV_3/FVC more sensitively identified reductions in terminal
20 expiratory flow and, accordingly, is able to diagnose mild airflow obstruction³⁷. Morris et al.
21 suggested that isolated reduction in FEV_3/FVC (without other spirometric abnormality) may
22 indicate early injury accompanied by hyperinflation and gas trapping^{37,38}. We found that isolated

1 FEV₃/FVC abnormality was not as diagnostic of either CT abnormalities or of COPD-related
2 patient-reported or functional outcomes (Tables 2 and 4).

3 Structural features in quantitative CT were correlated with FEV₁/FVC, FEV₁/FEV₆,
4 FEV₃/FEV₆ and FEV₃/FVC (e-Table 8). CT features that had strongest correlation with
5 spirometric ratios were small airway measures. These findings are similar to previous results
6 showing that airflow obstruction correlates with emphysema%, gas trapping% and airway
7 dimensions^{22,23,39}. Hersh et al. found that gas trapping%, a prominent indirect sign of small
8 airways disease, had a strong correlation with emphysema% and may fail to distinguish between
9 gas trapping caused by emphysema and by small airways disease^{24,34,40}. They described an index,
10 the E/I MLA, utilizing paired inspiratory and expiratory images, as a more reliable small airway
11 disease measure in smokers^{34,41}. Indeed, we found strong correlation between E/I MLA and all
12 spirometric airflow obstruction indices, including FEV₃/FEV₆ (r=-0.65) and FEV₃ %predicted
13 (r=-0.51). It is reasonable to infer that significant correlations of gas trapping and E/I MLA with
14 spirometric measurements are a consequence of hyperinflation due to small airways injury.

15 Lung elastic recoil loss causing expiratory airflow limitation results from emphysematous
16 lung destruction. However, presence of emphysema does not consistently elicit spirometric
17 airway obstruction. Hoesein et al. presented a longitudinal analysis showing that patients initially
18 exhibiting emphysema on CT but without airflow abnormality (defined as FEV₁/FVC <70%)
19 were prone to develop FEV₁ and FEV₁/FVC decline in follow-up studies⁴². Their results suggest
20 that FEV₁/FVC is not sensitive enough to diagnose mild structural changes until emphysema
21 severity exceeds a certain threshold. Our results indicate indirect CT measures of small airways
22 disease in a subset of subjects with otherwise normal spirometry (Table 2). We observed
23 significantly lower FEV₁ %predicted and FEV₁/FVC in participants with FEV₃/FEV₆ <LLN

1 compared to $FEV_3/FEV_6 \geq LLN$; however these values do not reach diagnostic criteria for
2 abnormality in FEV_1/FVC LLN (Table 2). This finding supports that there is lung structure and
3 function loss in the subgroup not yet recognized as abnormal by $FEV_1/FVC < LLN$ ¹⁷. Targeting
4 this undiagnosed population is of great importance, since functional small airways dysfunction is
5 associated with FEV_1 decline in non-obstructed smokers and small airway function improves
6 starting from the first week of smoking cessation in smokers who do not have airflow
7 obstruction^{43,44}.

8 Small airways have been regarded as the lung's "silent zone", because obstruction within
9 them causes little spirometric abnormality until obstruction is far advanced⁴⁵. Microstructural
10 studies in COPD showed that morphological small airways changes begin before
11 emphysematous destruction starts^{46,47}. In our study, subjects in whom FEV_3/FEV_6 was the sole
12 abnormality had significantly greater gas trapping%, E/I MLA and segmental airway wall area
13 without significantly greater emphysema% (Table 2, Figure 1). The reason for lack of
14 association between emphysema and FEV_3/FEV_6 abnormality may be that isolated FEV_3/FEV_6
15 abnormality diagnoses COPD in early stages before emphysematous destruction is detectable.
16 However, to confirm this suggestion further longitudinal analysis is required.

17 A vigorous physical effort and expiration time as long as 20 seconds are needed to
18 measure FVC accurately - hard to achieve in elderly and severely obstructed patients. A shorter
19 expiratory time causes FVC underestimation and FEV_1/FVC overestimation that may lead to
20 false negative interpretation in patients with mild airway obstruction^{12,48,49}. FEV_6 has the
21 advantage over FVC of being independent of forced expiration duration; previous studies have
22 shown that FEV_1/FEV_6 is a valid alternative to FEV_1/FVC to diagnose airflow obstruction⁴⁸⁻⁵⁴.
23 The present study supports previous observations showing that FEV_1/FEV_6 abnormality had

1 significantly stronger association with structural impairment (emphysema%, gas trapping%, E/I
2 MLA and increased WA%) and to COPD-related outcomes and 6MWD, compared to using
3 FEV₁/FVC abnormality (e-Tables 6 and 7).

4 Our results should be considered in light of its limitations and strengths. Distal airway
5 walls are dominantly composed of smooth muscle⁵⁵. Distal airway smooth muscle hypertrophy
6 contributes to bronchodilator reversibility in COPD⁵⁶. Varying degrees of smooth muscle
7 contraction may cause variation in airflow limitation. Smooth muscle contraction can be partially
8 reversed by inhaled bronchodilator administration. For this reason, using pre-bronchodilator
9 spirometry for detection of airflow obstruction may lead to overestimation of airflow obstruction.
10 This is the main reason for using post-bronchodilator spirometry in the current COPD
11 definition⁵⁷. In our analysis, we defined abnormality with regard to pre-bronchodilator LLN
12 criteria. From this perspective, unreversed smooth muscle contraction may be one reason why
13 we detected increased gas trapping and E/I MLA and impaired airway dimensions in
14 FEV₃/FEV₆≤LLN individuals. However, these structural differences remained significant after
15 excluding bronchodilator-responsive smokers from our analysis. Whether impairments defined in
16 this study precede full-blown COPD warrants further longitudinal analysis of this cohort.
17 Absence of a LLN criterion for post-bronchodilator FEV₃/FEV₆ prevents us from comparing
18 diagnostic capabilities of FEV₃/FEV₆ between pre- and post-bronchodilator measurements.
19 Studies that have evaluated diagnostic performance of different spirometric criteria have usually
20 made comparisons by accepting FEV₁/FVC (<LLN or <0.70) as “gold standard” in diagnosing
21 airflow obstruction^{3,58,59}. Our data demonstrates that this approach may be suboptimal for
22 diagnosing airflow obstruction, especially mild, early or small airways disease. We
23 acknowledge, though, that we have not demonstrated that a finding of isolated abnormality in

1 FEV₃/FEV₆ has prognostic or therapeutic implications. Further, like all spirometric measures,
2 day-to-day variability in lung function and measurement error can contribute to variability of
3 classification of those patients on the border between "normal" and mild abnormality.

4

5 Another point deserving consideration regards COPDGene inclusion strategy, which did
6 not exclude smokers with self-reported asthma history. This inclusion strategy may cause
7 over/misdiagnosis of patients who actually have asthma as having COPD or include those with
8 both asthma and COPD. Such smoking asthmatics may demonstrate all of the impairments in
9 functional, structural and quality of life indices observed in our study. We believe, spirometric
10 monitoring during CT scanning might improve standardization and decrease variability in the
11 quantitative CT measures. However, to our knowledge, this is not routinely done in quantitative
12 CT imaging and was not done in the studies of the COPDGene cohort. CT scans were, however,
13 performed by experienced staff, who coached participants to perform a maximal inspiratory
14 maneuver and a relaxed end-expiratory maneuver. Finally, testing new LLN equations in never-
15 smoker adults with available quantitative CT data would be advantageous to discriminate effects
16 of aging and smoking on structural and functional alterations. Nevertheless, we believe our
17 findings discriminate a subgroup with impairment in physiologic, functional, structural and
18 quality of life dimensions resembling COPD not identified by using FEV₁/FVC>LLN regardless
19 of whether these smokers are called asthma-COPD overlap syndrome, smoking asthmatics or
20 COPD.

21 In conclusion, increasing evidence supports presence of structural lung changes before
22 airflow obstruction becomes evident by routine spirometric criterion^{9,14-16}. Our findings, based
23 on the examination of the largest smoker population with available quantitative CT data,

1 demonstrate presence of structural lung changes before airflow obstruction becomes evident by
2 the FEV₁/FVC ratio. We report for the first time that, in those with normal FEV₁/FVC, low
3 FEV₃/FEV₆ is significantly associated with impaired CT measures, shorter 6-minute walk
4 distance, increased dyspnea perception and lower respiratory quality of life. It seems capable of
5 diagnosing spirometric abnormality at an early stage before marked emphysematous changes
6 start. Whether the FEV₁/FVC normal but FEV₃/FEV₆ abnormal population defined in this study
7 will show more rapid COPD progression is unknown. Longitudinal analysis of COPDGene and
8 other cohorts may provide the answer to this question.

9

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1 **Figure legends**

2 **Figure 1.** Comparison of functional, structural and COPD-related clinical indices of participants
3 with $FEV_3/FEV_6 \geq LLN$ (n=3,709) vs. $FEV_3/FEV_6 < LLN$ (n=677), among those without
4 clinically defined airflow obstruction by pre-bronchodilator $FEV_1/FVC \geq LLN$ (n=4,386).
5 Participants with $FEV_3/FEV_6 < LLN$ had significantly more impairment on quantitative CT (gas
6 trapping %, E/I MLA, segmental wall area), worse FEV_1 %pred and FEV_1/FVC , greater dyspnea
7 perception and SGRQ, and lower 6MWD. The central horizontal line on each box represents the
8 median, the ends of the boxes are 25 and 75 percentiles and the error bars 10 and 90%
9 percentiles. The lower and upper solid circles (●) represent minimum and maximum values in
10 each group, respectively. * denotes $P < 0.0001$; NS denotes $P > 0.05$. P values derived from the
11 two-sample t -test for normally distributed continuous variables, and the Mann-Whitney U test
12 for continuous non-normal data. Definition of abbreviations: LLN, lower limits of normal; FEV_1
13 %predicted, forced expiratory volume in 1 second expressed as percent predicted; FVC
14 %predicted, forced vital capacity expressed as percent predicted; E/I MLA, expiratory to
15 inspiratory ratio of mean lung attenuation; SGRQ, St. George's Respiratory Questionnaire;
16 mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance.
17 The BODE index includes body mass index (BMI), post-bronchodilator FEV_1 %predicted,
18 mMRC score, and the six-minute walk distance (6MWD).

19

1 **Figure 2.** Comparison of participants with pre-bronchodilator $FEV_1/FVC \geq LLN$ (n=4,386)
2 divided into subgroups of normal (dark grey bars, N=3709) and abnormal FEV_3/FEV_6 (light grey
3 bars, N=677) deemed to be abnormal by functional, structural and clinical outcomes. Bars
4 represent proportion of the population, the error bars lower and upper bounds for 95%
5 confidence interval. Statistical significance was determined by Pearson *chi-square*. * denotes $P <$
6 0.0001 ; NS denotes $P > 0.05$. Definition of abbreviations: n, number of subjects; FEV_1
7 %predicted, forced expiratory volume in 1 seconds expressed as percent predicted; mMRC,
8 modified Medical Research Council Dyspnea Scale; SGRQ, St. George's Respiratory
9 Questionnaire; 6MWD, 6 minute walking distance. The BODE index includes body mass index
10 (BMI), post-bronchodilator FEV_1 %predicted, mMRC score, and the six-minute walk distance
11 (6MWD).

12

1 Tables

2 **Table 1.** Characteristics of the study population with regard to FEV₁/FVC and FEV₃/FEV₆ LLN.

	FEV ₁ /FVC		FEV ₃ /FEV ₆	
	≥ LLN (n=4386)	< LLN (n=3467)	≥ LLN (n=3888)	< LLN (n=3965)
Age, years	56.9 (51 – 64)	62.7 (55 – 69) *	57.1 (51– 64)	61.9 (54.–69) [†]
Sex, Male %	51.7	55.8*	53.8	53.3
Race, Caucasian; African-American, %	64; 36	79; 21 *	66; 34	76; 24 [†]
Body mass index, kg/m ²	29.4 ± 6.0	27.8 ± 6.1*	29.6 ± 5.9	27.8 ± 6.1 [†]
Smoking history, pack.years [§]	36 (24 – 48)	45 (34 – 65)*	35 (23 – 48)	45 (33 – 65) [†]
Spirometry				
Post-BD FEV ₁ /FVC ≥70,%	86.8	8.6*	87.8	17.5 [†]
Pre-BD FEV ₁ %pred	88.7 ± 15.7	52.7 ± 22.2*	89.9 ± 15.3	56.0 ± 23.2 [†]
Pre-BD FEV ₃ %pred	95.2 ± 17.6	67.3 ± 23.4*	91.8 ± 15.1	78.7 ± 20.4 [†]
Pre-BD FEV ₆ %pred	91.0 ± 15.1	70.1 ± 20.5*	91.6 ± 14.7	72.1 ± 20.8 [†]
Pre-BD FVC %pred	91.1 ± 15.4	77.7 ± 20.7*	91.8 ± 15.1	78.7 ± 20.4 [†]
Post-BD FEV ₁ %pred	91.3 ± 15.8	56.9 ± 23.2*	92.4 ± 15.4	60.2 ± 24.0 [†]
Pre-BD FEV ₁ /FVC, %	75.3 (71.4 – 79.1)	52.8 (39.6 – 62.2)*	76.1 (72.1 – 79.6)	56.2 (41.8 – 65.0) [†]
Post-BD FEV ₁ /FVC, %	77.0 (72.8 –81.3)	53.9 (40.6 – 63.9)*	77.7 (73.3 – 81.7)	57.4 (42.5 – 67.2) [†]
Pre-BD FEV ₁ /FEV ₆ , %	78.4 (75.3 –81.5)	59.8 (48.3 – 67.9)*	79.0 (76.1 – 81.9)	62.7 (50.0 – 70.1) [†]
Pre-BD FEV ₃ /FVC, %	89.9 (87.4 –92.3)	75.0 (64.9 – 81.6)*	90.4 (87.8 – 92.6)	77.3 (66.6 – 83.9) [†]
Pre-BD FEV ₃ /FEV ₆ , %	93.3 (92.0 –94.5)	85.3 (78.5 – 89.1)*	93.6 (92.5 – 94.6)	86.7 (79.6 – 89.8) [†]
Quantitative CT indices				
LAA _{950insp} , % (emphysema%)	1.0 (0.4 – 2.5)	7.2 (2.2 – 18.7)*	1.1 (0.4 – 2.6)	5.3 (1.5 – 16.8) [†]
LAA _{856exp} , % (gas trapping%)	9.0 (4.2 – 15.6)	34.0 (18.0 – 53.6)*	8.9 (4.2 – 15.4)	30.0 (14.4 – 50.8) [†]
E/I MLA	0.83 (0.80-0.87)	0.91 (0.87-0.95)*	0.83 (0.79 – 0.87)	0.91 (0.86 – 0.94) [†]
WA _{segmental} , %	60.4 ± 3.0	62.5 ± 3.1*	60.3 ± 3.0	62.4 ± 3.2 [†]
HRQL and functional measures				

mMRC ^{††}	0.9 ± 1.2	1.9 ± 1.5*	0.8 ± 1.2	1.8 ± 1.5 [†]
SGRQ, total score	11.3 (3.5-29.5)	36.5 (18.2-54.5)*	10.5 (3.2 – 27.2)	34.8 (16.2 – 53.1) [†]
6MWD, meters [‡]	440 ± 109	376 ± 122*	445 ± 108	379 ± 122 [†]
BODE [‡]	0.9 ± 1.2	1.9 ± 1.5*	0.8 ± 1.2	1.8 ± 1.5 [†]

1 Data are mean ± standard deviation or median (IQR 25 – 75), as appropriate. *denotes P value <
2 0.0001 compared to FEV₁/FVC ≥LLN participants. [†] denotes P value < 0.0001 compared to
3 FEV₃/FEV₆ ≥LLN participants. [§] Data available for 7755 participants. ^{††} Data available for 7849
4 participants. [‡]Data available for 7756 participants. Statistical significance was determined by the
5 two-sample t -test for normally distributed continuous variables and the Mann-Whitney U test for
6 comparison of continuous non-normal data between FEV₃/FEV₆ ≥LLN vs. <LLN groups. Body
7 mass index, post-bronchodilator FEV₁%predicted, mMRC score, and 6MWD were integrated to
8 calculate the 10-point BODE index²⁷. Definition of abbreviations: pre-BD, pre-bronchodilator;
9 post-BD, post-bronchodilator; FEV₁ %predicted, forced expiratory volume in 1 second expressed
10 as percent predicted; FVC %predicted, forced vital capacity expressed as percent predicted;
11 FEV₃ %predicted, forced expiratory volume in 3 seconds expressed as percent predicted; FEV₆
12 %predicted, forced expiratory volume in 6 seconds expressed as percent predicted; LAA_{-950insp},
13 total percentage of all lung voxels less than or equal to –950 Hounsfield Units (HU) on end-
14 inspiratory scans, commonly called Emphysema%; LAA_{-856exp}, total percentage of all lung
15 voxels less than or equal to –856 Hounsfield Units (HU) on expiratory scans, commonly called
16 Gas Trapping%; E/I MLA, expiratory to inspiratory ratio of mean lung attenuation; WA, wall
17 area; HRQL, health-related quality of life; SGRQ, St. George’s Respiratory Questionnaire;
18 6MWD, 6 minute walking distance; mMRC, modified Medical Research Council Dyspnea
19 Scale; COPD, chronic obstructive pulmonary disease.

Table 2. Functional, structural and clinical characteristics of participants with pre-bronchodilator FEV₁/FVC \geq LLN divided into subgroups of normal and abnormal FEV₃/FEV₆ and FEV₃/FVC.

	FEV1/FVC \geq LLN						
	TOTAL	FEV ₃ /FEV ₆ \geq LLN	FEV ₃ /FEV ₆ <LLN	P [†]	FEV ₃ /FVC \geq LLN	FEV ₃ /FVC <LLN	P ^{††}
n, (%)	4386 (100)	3709 (84.6)	677 (15.4)	-	4074 (92.9)	312 (7.1)	-
Age, years	58.0 \pm 8.7	58.0 \pm 8.7	57.9 \pm 8.6	0.78	58.2 \pm 8.7	56.2 \pm 7.6	< 0.0001
Sex, Male %	51.7	53.0	44.9	< 0.0001	51.2	58.0	0.02
Smoking history, pack.years	35.7 (23.7 – 48.3)	35.1 (23.0 – 48.0)	38.0 (27.7 – 52.8)	< 0.0001	35.5 (23.3 – 48.1)	36.7 (25.9 – 48.8)	0.15
FEV ₁ , % pred	88.7 \pm 15.7	90.4 \pm 15.1	79.1 \pm 15.6	< 0.0001	89.0 \pm 15.7	85.0 \pm 14.7	< 0.0001
FEV ₃ , % pred	95.2 \pm 17.6	96.5 \pm 17.2	88.3 \pm 18.0	< 0.0001	95.6 \pm 17.6	90.7 \pm 16.1	< 0.0001
FEV ₆ , % pred	91.0 \pm 15.1	91.8 \pm 14.6	86.7 \pm 16.8	< 0.0001	91.1 \pm 15.1	89.8 \pm 14.9	< 0.0001
FVC, % pred	91.1 \pm 15.4	91.8 \pm 14.9	87.4 \pm 17.1	< 0.0001	90.8 \pm 15.3	94.8 \pm 16.1	< 0.0001
FEV ₁ /FVC, %	75.3 (71.4 – 79.1)	76.4 (72.7 – 79.8)	70.2 (68.2 – 72.1)	< 0.0001	75.8 (72.0 – 79.4)	69.5 (67.8 – 71.4)	< 0.0001
FEV ₃ /FEV ₆ , %	93.3 (92.0 – 94.5)	93.6 (92.6 – 94.7)	90.6 (89.8 – 91.4)	< 0.0001	93.4 (92.1 – 94.6)	91.7 (90.6 – 92.7)	< 0.0001
FEV ₃ /FVC, %	89.9 (87.5 – 92.3)	90.6 (88.2 – 92.6)	86.9 (84.9 – 88.4)	< 0.0001	90.2 (88.0 – 92.5)	83.8 (82.3 – 85.4)	< 0.0001
LAA _{-950insp} , %	0.98 (0.39 – 2.53)	0.97 (0.40 – 2.47)	1.03 (0.38 – 2.85)	0.18	0.98 (0.39 – 2.48)	1.08 (0.41 – 3.09)	0.12
LAA _{-856exp} , %	9.0 (4.2 – 15.6)	8.6 (4.0 – 15.1)	10.8 (5.3 – 19.6)	< 0.0001	9.0 (4.1-15.6)	10.0 (5.2 – 15.4)	0.09
E/I MLA	0.83 (0.80 – 0.87)	0.83 (0.79 – 0.87)	0.85 (0.81 – 0.89)	< 0.0001	0.83 (0.79 – 0.87)	0.84 (0.80 – 0.87)	0.04
WA _{segmental} , %	60.4 \pm 3.0	60.3 \pm 3.0	61.4 \pm 3.2	< 0.0001	60.4 \pm 3.1	60.4 \pm 3.0	0.87
SGRQ total score	11.3 (3.5 \pm 29.5)	10.4 (3.0 – 27.0)	19.9 (6.2 – 38.3)	< 0.0001	11.1 (3.5 – 29.1)	15.0 (3.8 – 34.7)	0.01
mMRC*	0.88 \pm 1.25	0.8 \pm 1.2	1.2 \pm 1.4	< 0.0001	0.9 \pm 1.2	1.0 \pm 1.3	0.05
6MWD, meters [‡]	440 \pm 109	444 \pm 108	415 \pm 116	< 0.0001	439 \pm 109	447 \pm 118	0.22
BODE index [‡]	0.6 \pm 1.0	0.5 \pm 0.9	0.9 \pm 1.3	< 0.0001	0.6 \pm 1.0	0.7 \pm 1.1	0.09

Data are expressed as mean \pm standard deviation or median (IQR 25 – 75) as appropriate.

† Univariate comparison between $FEV_3/FEV_6 \geq LLN$ vs. $FEV_3/FEV_6 < LLN$. †† Univariate comparison between $FEV_3/FVC \geq LLN$ vs. $FEV_3/FVC < LLN$. * Data available for 4385 participants. ‡ Data available for 4364 participants.

Statistical significance was determined by the two-sample *t*-test for normally distributed continuous variables, the Mann-Whitney *U* test for comparison of continuous non-normal data between $FEV_3/FEV_6 \geq LLN$ vs. $< LLN$ and $FEV_3/FVC \geq LLN$ vs. $< LLN$ groups.

Definition of abbreviations: n, number of subjects; FEV_1 %predicted, forced expiratory volume in 1 second expressed as percent predicted; FEV_3 %predicted, forced expiratory volume in 3 seconds expressed as percent predicted; FEV_6 %predicted, forced expiratory volume in 6 seconds expressed as percent predicted; FVC %predicted, forced vital capacity expressed as percent predicted; $LAA_{-950insp}$, total percentage of all lung voxels less than or equal to -950 Hounsfield Units (HU) on end-inspiratory scans, commonly called Emphysema%; $LAA_{-856exp}$, total percentage of all lung voxels less than or equal to -856 Hounsfield Units (HU) on expiratory scans, commonly called Gas Trapping%; E/I MLA, expiratory to inspiratory ratio of mean lung attenuation; WA, wall area; SGRQ, St. George's Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance. The BODE index includes body mass index, post-bronchodilator FEV_1 %predicted, mMRC score, and the 6MWD.

Table 3. General linear regression models of difference in quantitative CT indices, quality of life and 6 minute walk distance in participants with FEV₃/FEV₆ <LLN among those with FEV₁/FVC ≥LLN (n=4386).

	Gas Trapping% ^{†1}	Wall area% ^{†1}	E/I MLA% ^{†1}	SGRQ ^{†2}	6MWD ^{†2}	mMRC ²	BODE ²
	% difference	% difference	% difference	% difference	% difference	OR	OR
FEV₃/FEV₆ <LLN vs.							
FEV₃/FEV₆ ≥LLN*	27.5	1.8	2.0	40.8	-7.2	1.6	2.8
95% CI	18.1- 37.8	1.4 - 2.2	1.6 - 2.4	28.0 - 55.0	-9.4 - (-5.0)	1.4 - 1.9	2.1 - 3.7
e^β	1.275	1.018	1.020	1.408	0.928	-	-
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

^{†1} Gas trapping%, E/I MLA, wall area%, SGRQ and 6MWD were natural log transformed. The displayed coefficients (% difference and CI 95%) are back-transformed regression coefficients that correspond to the relative differences between the two groups in percent in ratios. For example, for gas trapping %, the expected mean gas trapping % of FEV₃/FEV₆ <LLN group is higher than the one of FEV₃/FEV₆ ≥ LLN by 27.5%. OR indicates the relative odds increase for a higher score of mMRC or BODE between the two groups. For example, the estimated odds of having a one unit higher score of mMRC dyspnea score (worsening from 0 to 4) for FEV₃/FEV₆ <LLN is 1.6 of the odds compared with FEV₃/FEV₆ ≥ LLN. BODE index scores were categorized into four severity stages; BODE stage 1 to 4 with scores of 0-2, 3-4, 5-6 and 7-10, respectively.

* FEV₃/FEV₆ ≥ LLN data was used as the reference category. ¹ Models controlled for sex, age, race, body mass index, smoking history (pack-years of smoking) and CT scanner type. ² Models controlled for sex, age, race, body mass index and smoking history (pack-years of smoking).

Definition of abbreviations: E/I MLA, expiratory to inspiratory mean lung attenuation; SGRQ, St. George Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; OR, odds ratio; CI 95%, exponential (back-transformed) 95% confidence intervals, e^β: exponential (back-transformed) regression coefficients.

Table 4. General linear regression models of difference in quantitative CT indices and quality of life in participants with FEV₃/FVC <LLN among those with FEV₁/FVC ≥LLN (n=4386).

	Gas Trapping%^{†1}	E/I MLA%^{†1}	SGRQ^{†2}	mMRC²	BODE²
	% difference	% difference	% difference	OR	OR
FEV₃/FVC <LLN vs.					
FEV₃/FVC ≥LLN*	18.8	1.2	15.4	1.3	1.8
95% CI	6.6 - 32.3	0.4 - 2.0	0.9 - 32.1	1.03 - 1.61	1.2 - 2.6
e^β	1.188	1.012	1.154	-	-
P	0.002	< 0.0001	0.037	0.029	0.004

^{†1} Gas trapping%, E/I MLA, and SGRQ were natural log transformed. The displayed coefficients (% difference and CI 95%) are back-transformed regression coefficients that correspond to the relative differences between the two groups in percent in ratios. For example, for gas trapping%, the expected mean gas trapping % of FEV₃/FEV₆ <LLN group is higher than the one of FEV₃/FEV₆ ≥ LLN by 18.8%. OR indicates the relative odds increase for a higher score of mMRC or BODE between the two groups. For example, the estimated odds of having a one unit higher score of mMRC dyspnea score (worsening from 0 to 4) for FEV₃/FEV₆ <LLN is 1.3 of the odds compared with FEV₃/FEV₆ ≥ LLN. BODE index scores were categorized into four severity stages; BODE stage 1 to 4 with scores of 0-2, 3-4, 5-6 and 7-10, respectively.

* FEV₃/FVC ≥ LLN data was used as the reference category. ¹ Models controlled for sex, age, race, body mass index, smoking history (pack-years of smoking) and CT scanner type. ² Models controlled for sex, age, race, body mass index and smoking history (pack-years of smoking).

Definition of abbreviations: E/I MLA, expiratory to inspiratory mean lung attenuation; SGRQ, St. George Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; OR, odds ratio; CI 95%, exponential (back-transformed) 95% confidence intervals, e^β: exponential (back-transformed) regression coefficients.

Abbreviations list

BMI	body mass index
BODE	BODE index includes body mass index, post-bronchodilator FEV ₁ %predicted, mMRC score, and 6MWD.
CI	confidence intervals
COPD	chronic obstructive pulmonary disease
CT	computed tomography
E/I MLA	expiratory to inspiratory ratio of mean lung attenuation on quantitative CT scan
FEV ₁ %predicted	forced expiratory volume in 1 second expressed as percent predicted
FEV ₃ %predicted	forced expiratory volume in 3 seconds expressed as percent predicted
FEV ₆ %predicted	forced expiratory volume in 6 seconds expressed as percent predicted
FVC %predicted	forced vital capacity expressed as percent predicted
HRQL	health-related quality of life
HU	Hounsfield Units
LAA _{-950insp}	total percentage of all lung voxels less than or equal to -950 Hounsfield Units (HU) on end-inspiratory scans, commonly called emphysema %
LAA _{-856exp}	total percentage of all lung voxels less than or equal to -856 Hounsfield Units (HU) on expiratory scans, commonly called gas trapping %
LLN	Lower limits of normal
mMRC	modified Medical Research Council Dyspnea Scale
NHANES-III	Third National Health and Nutrition Examination Survey
OR	odds ratio
SGRQ	St. George's Respiratory Questionnaire

6MWD

Six-minute walking distance

WA %:

wall area %

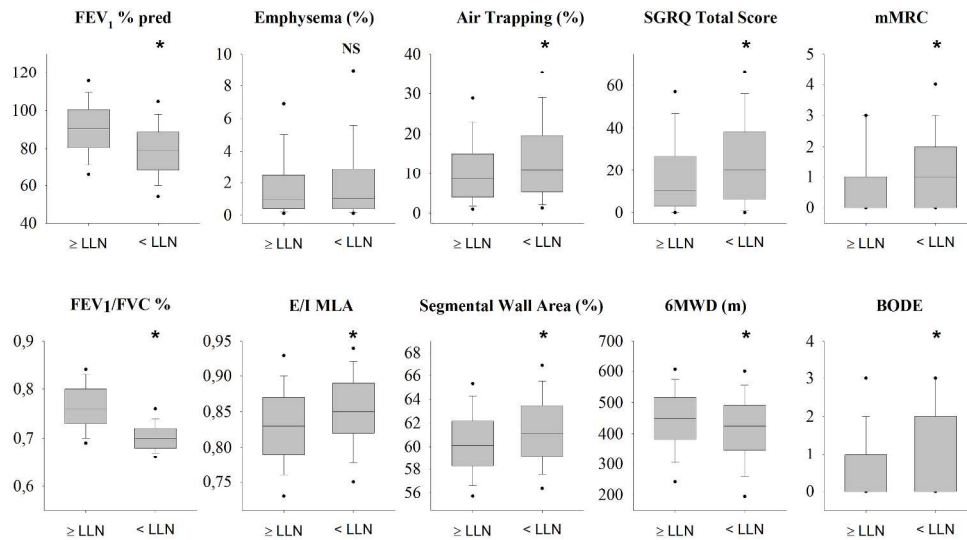


Figure 1. Comparison of functional, structural and COPD-related clinical indices of participants with FEV₃/FEV₆ ≥LLN (n=3,709) vs. FEV₃/FEV₆ <LLN (n=677), among those without clinically defined airflow obstruction by pre-bronchodilator FEV₁/FVC ≥LLN (n=4,386). Participants with FEV₃/FEV₆ <LLN had significantly more impairment on quantitative CT (gas trapping %, E/I MLA, segmental wall area), worse FEV₁ %pred and FEV₁/FVC, greater dyspnea perception and SGRQ, and lower 6MWD. The central horizontal line on each box represents the median, the ends of the boxes are 25 and 75 percentiles and the error bars 10 and 90% percentiles. The lower and upper solid circles (●) represent minimum and maximum values in each group, respectively. * denotes P < 0.0001; NS denotes P > 0.05. P values derived from the two-sample t-test for normally distributed continuous variables, and the Mann-Whitney U test for continuous non-normal data. Definition of abbreviations: LLN, lower limits of normal; FEV₁ %predicted, forced expiratory volume in 1 second expressed as percent predicted; FVC %predicted, forced vital capacity expressed as percent predicted; E/I MLA, expiratory to inspiratory ratio of mean lung attenuation; SGRQ, St. George's Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance. The BODE index includes body mass index (BMI), post-bronchodilator FEV₁%predicted, mMRC score, and the six-minute walk distance (6MWD).
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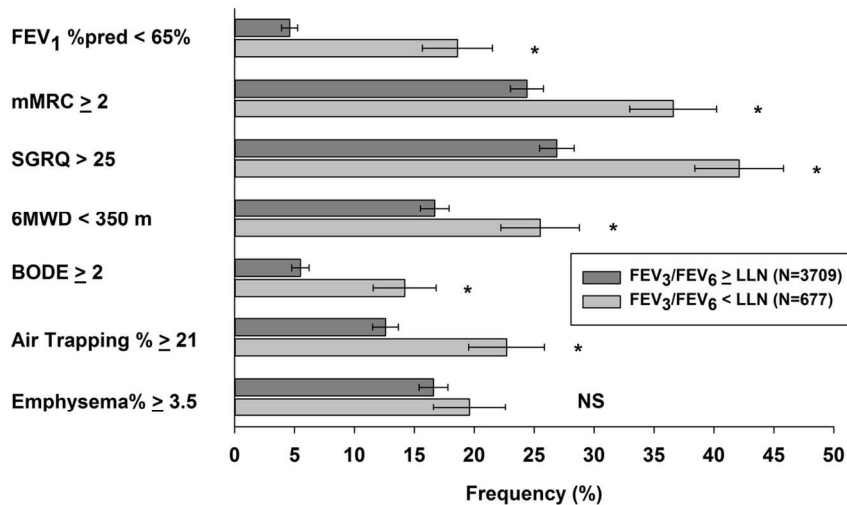


Figure 2. Comparison of participants with pre-bronchodilator FEV₁/FVC ≥ LLN (n=4,386) divided into subgroups of normal (dark grey bars, N=3709) and abnormal FEV₃/FEV₆ (light grey bars, N=677) deemed to be abnormal by functional, structural and clinical outcomes. Bars represent proportion of the population, the error bars lower and upper bounds for 95% confidence interval. Statistical significance was determined by Pearson chi-square. * denotes P < 0.0001; NS denotes P > 0.05. Definition of abbreviations: n, number of subjects; FEV₁ %predicted, forced expiratory volume in 1 seconds expressed as percent predicted; mMRC, modified Medical Research Council Dyspnea Scale; SGRQ, St. George's Respiratory Questionnaire; 6MWD, 6 minute walking distance. The BODE index includes body mass index (BMI), post-bronchodilator FEV₁%predicted, mMRC score, and the six-minute walk distance (6MWD).

126x74mm (300 x 300 DPI)

1 **A novel spirometric measure identifies mild chronic obstructive pulmonary disease**
2 **unidentified by standard criteria**

3

4 **-- Online Data Supplement --**

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17

1 **Methods**

2 *Inclusion and exclusion criteria*

3 10,311 male and female non-Hispanic White and African-American participants, 45 to 80
4 years old, with at least 10 pack-years of smoking history were included in the study. Participants
5 with incomplete quantitative CT data (n=1,704), or with incomplete measurements in any of the
6 relevant spirometric data (n=591), and with incomplete measurements in both spirometry and CT
7 (n=163) were excluded, reducing the study population to 7,853 in the present study¹.

8

9 *Patient-reported outcomes and 6 minute walk distance*

10 Participants' overall health-related quality of life, symptoms and impact of disease were
11 assessed by: St. George's Respiratory Questionnaire (SGRQ, scores ranging from 0-100; where a
12 higher score indicates worse health status)²; the modified Medical Research Council Dyspnea
13 Scale (mMRC, ranging from 0 to 4³; the multidimensional BODE index (ranging from 0 to 10,
14 higher score indicating worse outcome)⁴. The BODE index uses body mass index (BMI), post-
15 bronchodilator FEV₁%predicted, mMRC score, and the six-minute walk distance (6MWD).
16 BODE index scores were categorized into four severity stages; BODE stage 1 to 4 with scores of
17 0-2, 3-4, 5-6 and 7-10, respectively. Six-minute walking test was performed according to ATS
18 standards⁵.

19

20 *Spirometry, quality control and definition of normality*

21 Spirometry tests were performed by using an ultrasound-based spirometer (NDD, EasyOne
22 Spirometer Medizintechnik AG, Zurich, Switzerland) before and after administration of 180 mcg
23 of short-acting β_2 -agonist (albuterol) in accordance with the ATS recommendations⁶.

1 Spirometric measurements were reviewed and graded (ranging from 0 – 4) by an automated
2 quality assessment software package and by a centralized quality control process established for
3 the COPDGene project (Grade 4: fully met ATS criteria, reproducible to within 50 ml, Grade 3:
4 fully met ATS criteria, reproducible to between 50-100 ml, Grade 2: fully met ATS criteria,
5 reproducible between 100 and 150 ml; Grade 1: partly meeting ATS criteria and/or reproducible
6 between 150-200 ml; Grade 0: failure to meet ATS criteria and/or reproducible greater than 200
7 ml)⁷. Mean primary quality control grades for pre-bronchodilator FEV₁ and FVC measurements
8 were 3.44 ± 0.90 and 3.21 ± 1.03, respectively in the selected group for the present study.

9 Best “test” was defined as the maneuver with the largest sum of FVC and FEV₁. For our
10 analysis, values of the best pre-bronchodilator FEV₁, FVC, FEV₃, and FEV₆ were chosen;
11 percent predicted values were calculated from the NHANES-III reference equations (n=5,880)^{8,9}.
12 The linear iteratively-derived equations of Hansen et al.¹⁰ were used to determine LLN (lowest
13 5th percentile) for pre-bronchodilator FEV₁/FVC, FEV₁/FEV₆, FEV₃/FEV₆ and FEV₃/FVC.

14

15 *Quantitative CT analysis and definition of indices*

16 CT scans were acquired by using a 16- or 64-detector CT scanner at full inspiration and
17 after tidal expiration according to a standardized protocol¹; image acquisition and analysis
18 procedures have been previously described^{11,12}. Emphysema% was assessed by using 3D Slicer
19 (<http://www.slicer.org>). Airway disease was assessed by wall area percentage of segmental
20 airways WA% = (bronchus outer area - airway luminal area) / (bronchus outer area) using
21 ‘Pulmonary Workstation Plus’ software (VIDA Diagnostics, Coralville, IA, U.S.A.)¹³. The CT
22 scans were planned in the COPDGene protocol to be done last on the visit day, after all
23 pulmonary function and other functional tests and symptom assessment questionnaires.

1 *Statistical Analyses*

2 Means and standard deviations were used to describe continuous variables and
3 proportions were used for categorical variables. For non-normal data, medians and interquartile
4 ranges were reported (25th and 75th percentiles) (Tables 1, 2, e-Tables 3 and 6). A chi-square test
5 was used to compare proportions between two groups and a two-sample t-test or one-way
6 ANOVA were used for continuous outcome variables. For non-normal data, the Kruskal-Wallis
7 test was used (Tables 1, 2, and e-Tables 3 and 6). Pairwise comparisons were followed by
8 Tukey's *post hoc* test, to adjust for an overall type I error rate of 5%. Pearson and Spearman
9 coefficients were used to estimate the linear relationships among spirometry, quantitative CT
10 parameters and disease-related outcomes (e-Table 8). In order to assess the relationship between
11 spirometric measurements (independent variable), and quantitative CT measures, SGRQ and
12 6MWD (outcome variables), a multiple general linear regression model was used for each
13 outcome variable, using age, sex, race, smoking history, BMI and CT scanner type (only for CT
14 measures) as covariates. Other covariates were included in the model where the P-value was less
15 than 0.10. The natural log transformation was used for quantitative CT measures, SGRQ and
16 6MWD to ensure validity of model assumptions. The consequent regression coefficients (β) were
17 back transformed for easier interpretation. Various reference categories were used for
18 comparisons of spirometric variables. Compared to a reference category, the relative difference
19 for dependent outcome variables was determined by $(e^{\beta} - 1) \times 100$ (%), while holding all other
20 predictors constant. For example, an exponentiated β coefficient (e^{β}) of 1.43 for the outcome
21 LAA_{.950insp} corresponds to a 43% greater mean of LAA_{.950insp} in patients with FEV₁/FEV₆ <LLN
22 compared with the reference group (in this case, those with both FEV₁/FEV₆ \geq LLN and
23 FEV₁/FVC \geq LLN). For ordinal outcomes (BODE and mMRC), the proportional odds model

1 was used (Tables 3, 4, and e-Tables 1, 2, 4, 7). P-values less than 0.05 were considered
2 statistically significant. For all statistical models, residual analyses were conducted to check the
3 model assumptions. Statistical analyses were performed with SPSS v22.0 (Armonk, NY: IBM
4 Corp.).

5 Spirometric, demographic, radiologic and clinical characteristics were compared in three
6 ways. Firstly, participants were characterized by FEV₃/FEV₆ above and below LLN (Tables 1-3,
7 e-Tables 1 and 2). Secondly, participants with FEV₃/FVC <LLN were compared with those in
8 whom FEV₃/FVC ≥LLN (Tables 2 and 4). These analyses were performed separately for the
9 total study population (7,853) and the subgroup in whom FEV₁/FVC ≥LLN (4,386). Finally,
10 demographic, spirometric, radiologic and clinical characteristics were compared in a) FEV₁/FVC
11 and FEV₃/FEV₆ concordant and discordant groups (e-Tables 3 and 4), and b) FEV₁/FVC and
12 FEV₁/FEV₆ concordant and discordant groups (e-Tables 6 and 7).

13

14 **Results**

15 *Correlates of FEV₁/FVC and FEV₃/FEV₆ abnormality in the overall group*

16 After adjustment for sex, age, race, body mass index, smoking history and CT scanner
17 type (only for quantitative CT parameters), with the exception of higher contribution of
18 FEV₁/FVC abnormality to predict extent of emphysema and gas trapping, FEV₃/FEV₆ and
19 FEV₁/FVC abnormality had significant and similar associations with E/I MLA, segmental airway
20 dimensions in quantitative CT, SGRQ, mMRC, 6MWD and BODE in the entire study population
21 (e-Table 1). In participants with FEV₃/FEV₆ abnormality (independent of obstruction determined
22 by FEV₁/FVC), emphysema% was 228.0% greater, gas trapping% was 152.3% greater, WA%
23 was 3.9% greater and E/I MLA was 6.6% greater, compared to those with FEV₃/FEV₆ >LLN.

1 FEV₃/FEV₆ abnormality was also associated with worse clinical outcomes: 136.0% greater
2 SGRQ score and 16.5% shorter 6MWD (e-Table 1) in the entire study population. These subjects
3 were also more likely to be in a higher mMRC category (adjusted OR: 3.9, P<0.0001) and
4 BODE index (adjusted OR: 10.5, P<0.0001) compared to those with normal FEV₃/FEV₆.

5
6 *FEV₃/FEV₆ is more sensitive in detecting structural, functional and patient-reported outcomes*
7 *than FEV₁/FVC*

8 Demographic, radiological, functional and patient-reported outcomes were compared
9 among patients with one or more abnormality in FEV₃/FEV₆ and FEV₁/FVC and those with
10 normal spirometry (e-Table 3). Significant differences were detected among these 4 groups.
11 47.2% of participants were normal in both FEV₃/FEV₆ and FEV₁/FVC, while 41.9% had
12 abnormality in both FEV₃/FEV₆ and FEV₁/FVC. Importantly, a substantial fraction of the study
13 population (n=677 participants, 8.6%) were abnormal only in FEV₃/FEV₆. These participants had
14 lower FEV₁% pred, FEV₃% pred, FEV₁/FVC %, FEV₃/FEV₆ %, higher gas trapping%, E/I MLA,
15 segmental WA%, as well as worse 6MWD, mMRC, SGRQ and BODE indices compared with
16 those normal in both FEV₃/FEV₆ and FEV₁/FVC. Notably, the extent of emphysema was not
17 statistically different from those normal in both FEV₃/FEV₆ and FEV₁/FVC. Fewer subjects were
18 abnormal in only FEV₁/FVC (n=179, 2.3%). These 179 participants had lower spirometric values
19 and significantly impaired quantitative CT indices including emphysema, however the mMRC,
20 SGRQ, 6MWD and BODE indices were not statistically different from those normal in both
21 FEV₃/FEV₆ and FEV₁/FVC. Participants with isolated FEV₁/FVC abnormality had significantly
22 greater emphysema and gas trapping, smaller segmental WA% and shorter 6MWD compared to
23 those abnormal with FEV₃/FEV₆. Participants with abnormality in both FEV₃/FEV₆ and

1 FEV₁/FVC had significant impairments in all of the spirometric, structural, functional and
2 quality of life indices compared to patients with either one abnormality in FEV₃/FEV₆ or
3 FEV₁/FVC (e-Table 3).

4 After adjustment for sex, age, race, body mass index, smoking history and CT scanner
5 type, in participants with isolated FEV₃/FEV₆ abnormality, emphysema% was 11.3% greater,
6 gas trapping % was 24.1% greater, WA% was 1.8% greater and E/I MLA was 2.3% greater
7 compared to those normal in both FEV₃/FEV₆ and FEV₁/FVC. Isolated FEV₃/FEV₆ abnormality
8 was also associated with worse clinical outcomes: 40.1% greater SGRQ score and 6.9% shorter
9 6MWD (e-Table 4). These participants were also more likely to be in a higher mMRC dyspnea
10 category (adjusted OR:1.6, P<0.0001) and BODE index category (adjusted OR:2.5, P<0.0001)
11 compared to those with normal FEV₃/FEV₆ and FEV₁/FVC (e-Table 4). Participants with only
12 FEV₁/FVC abnormality had significant associations with all of the radiologic and patient-
13 reported indices except 6MWD. Although isolated FEV₁/FVC abnormality subjects had stronger
14 associations for emphysema and gas trapping extent compared to those with isolated FEV₃/FEV₆
15 abnormality, those with isolated FEV₃/FEV₆ abnormality had stronger associations with E/I
16 MLA –the more sensitive CT parameter related to small airways dysfunction, as well as with
17 functional exercise capacity, quality of life and mMRC compared to those with isolated
18 FEV₁/FVC abnormality. Expectedly, participants with airflow obstruction assessed by both
19 FEV₃/FEV₆ and FEV₁/FVC had the highest increase in means of all quantitative CT parameters,
20 SGRQ scores and impairment in 6MWD. They also had the highest risk to be in a higher
21 category for mMRC score and BODE index (e-Table 4).

22

23 *Impairments in subjects with FEV₃/FEV₆ abnormality and FEV₁/FVC ≥LLN with regard to sex*

1 When sex was analyzed to assess whether it was a significant determinant for functional,
2 structural and clinical impairments impairments seen in Figure 2, the only sex-related difference
3 was in the more frequent presence of pathologically important emphysema in males (e-Table 5).
4 Recent results demonstrated that men display greater radiographic emphysema extent than
5 women in the overall COPDGene population¹⁴. Our results establish that there is also a sex-
6 specific emphysema pattern in the FEV₁/FVC normal smoker population.

7
8 *FEV₁/FEV₆ is more sensitive in detecting structural, functional and patient-reported outcomes*
9 *than FEV₁/FVC.*

10 Radiological, functional and patient-reported outcomes were compared among patients
11 with one or more abnormality in FEV₁/FEV₆ and FEV₁/FVC and those with normal spirometry
12 (e-Table 6). 52.7% of participants were normal in both FEV₁/FEV₆ and FEV₁/FVC, while 40.5%
13 had abnormality in both FEV₁/FEV₆ and FEV₁/FVC. 244 participants (3.1%) were abnormal in
14 only FEV₁/FEV₆, and 283 participants (3.6%) were abnormal in only FEV₁/FVC. Spirometric,
15 functional and quality of life indices were significantly different among these 4 groups (e-Table
16 6).

17 The presence of only FEV₁/FVC <LLN was associated with lower FEV₁ %pred,
18 emphysema% and gas trapping%, but E/I MLA, segmental wall area %, SGRQ, mMRC,
19 6MWD, and BODE were not different compared with those in whom both FEV₁/FVC and
20 FEV₁/FEV₆ were normal (e-Table 6). After adjustment for sex, age, race, body mass index,
21 smoking history and CT scanner type (for only quantitative CT parameters), general linear
22 regression models showed that those with abnormality in both FEV₁/FEV₆ and FEV₁/FVC had
23 the most impaired quantitative CT, SGRQ scores and 6MWD. They also had the highest risk for

1 greater mMRC and BODE index categories (e-Table 7). In addition, participants with isolated
2 FEV₁/FEV₆ abnormality compared with those normal in both FEV₁/FEV₆ and FEV₁/FVC were
3 associated with poorer spirometric measurements (FEV₁ %pred, FEV₁/FVC, FEV₁/FEV₆),
4 increased abnormality in quantitative CT (emphysema%, gas trapping%, E/I MLA, and WA%),
5 poorer functional and patient reported (SGRQ, mMRC, 6MWD, BODE) outcomes (e-Table 6).
6 The general linear regression models showed that, compared to participants normal in both
7 FEV₁/FEV₆ and FEV₁/FVC spirometry, participants with FEV₁/FEV₆ as the sole abnormality had
8 a 43.0% greater emphysema extent, 62.5% greater gas trapping%, 50.3% greater SGRQ score,
9 and 8.3% shorter 6MWD (e-Table 7). Participants with FEV₁/FEV₆ as the sole abnormality had
10 an increased risk to be in the higher mMRC and BODE category.

11 Participants in whom only FEV₁/FEV₆ was abnormal had lower FEV₁ %pred,
12 FEV₁/FVC, FEV₁/FEV₆ and 6MWD, greater BODE score, wall area% and E/I MLA compared
13 with the sole abnormality based on FEV₁/FVC (e-Table 6). Importantly, in general linear
14 regression models individuals with FEV₁/FEV₆ <LLN had worse emphysema%, gas trapping%,
15 WA%, E/I MLA, SGRQ, 6MWD, mMRC and BODE than those with only FEV₁/FVC <LLN (e-
16 Table 7). Therefore, FEV₁/FEV₆ <LLN is better able to define clinically-relevant abnormality
17 than FEV₁/FVC <LLN.

18

19

20 *Spirometric indices are correlated with quantitative CT and COPD-related outcomes*

21 In univariate correlation analysis, measures of structural abnormality in quantitative CT
22 were inversely related to each of the spirometric indices investigated: FEV₁/FVC, FEV₁/FEV₆,
23 FEV₃/FEV₆ and FEV₃/FVC (e-Table 8). Among CT indices, gas trapping% showed the strongest

1 inverse correlations with spirometric measurements (correlation coefficient of gas trapping %
2 with FEV₁/FVC %, FEV₁/FEV₆ %, FEV₃/FEV₆ %, FEV₃/FVC % ranges from - 0.68 to -0.72,
3 P<0.0001). E/I MLA had moderate inverse correlations with spirometry (correlation coefficient
4 of E/I MLA with spirometric ratios range from -0.60 to -0.67, P<0.0001), while segmental WA%
5 had weak-to-moderate inverse correlations with spirometric measures (r ranges from -0.27 to -
6 0.36, P<0.0001). Among disease-related outcomes, BODE had the highest correlation with the
7 spirometry, likely because it includes FEV₁ %predicted.

8

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18

19

e-Table 1. General linear regression models of difference in quantitative CT indices, quality of life and 6 minute walk distance in participants with FEV₃/FEV₆ <LLN and FEV₁/FVC <LLN groups for the entire cohort (n=7853).

	Emphysema% ^{†1}	Gas trapping% ^{†1}	Wall area % ^{†1}	E/I MLA % ^{†1}	SGRQ ^{†2}	6MWD ^{†2}	mMRC ²	BODE ²
	% difference	% difference	% difference	% difference	% difference	% difference	OR	OR
FEV₁/FVC <LLN vs.								
FEV₁/FVC ≥ LLN[*]	321.0	184.0	4.0	7.1	146.0	-16.8	4.2	11.4
95% CI	297 - 346	172 - 196	3.8 – 4.2	6.8 – 7.4	134 - 159	-18.3–(-15.32)	3.9 – 4.6	9.9 – 13.1
e^β	4.21	2.84	1.040	1.071	2.46	0.832	-	-
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
FEV₃/FEV₆ <LLN vs.								
FEV₃/FEV₆ ≥ LLN[‡]	228.0	152.3	3.9	6.6	136.0	-16.5	3.9	10.5
95% CI	208.8 - 248.3	141.9 - 163.0	3.6 - 4.1	6.3 - 6.9	124.4 - 148.1	-17.9 - (-15.0)	3.5 - 4.2	9.0- 12.2
e^β	3.28	2.523	1.039	1.066	2.360	0.835	-	-
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Definition of abbreviations: E/I MLA, expiratory to inspiratory mean lung attenuation; SGRQ, St. George Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; OR, odds ratio; CI 95%, exponential (back-transformed) 95% confidence intervals, e^β: exponential (back-transformed) regression coefficients. BODE index scores were categorized into four severity stages; BODE stage 1 to 4 with scores of 0-2, 3-4, 5-6 and 7-10, respectively.

[†] The emphysema%, gas trapping%, E/I MLA, wall area %, SGRQ and 6MWD were natural log transformed. The displayed coefficients (% difference and CI 95%) are back-transformed regression coefficients that correspond to the relative differences between the two groups in percent in ratios. For example, for emphysema%, the expected mean emphysema% of FEV₃/FEV₆ <LLN group is higher than the one of FEV₃/FEV₆ ≥ LLN by 228.0%. OR indicates the relative odds increase for a higher score of mMRC or BODE between the two groups. For example, the estimated odds of having a one unit higher score of mMRC dyspnea score (worsening from 0 to 4) for FEV₃/FEV₆ <LLN is 3.9 of the odds compared with FEV₃/FEV₆ ≥ LLN.

* FEV₁/FVC ≥ LLN data was used as the reference category. [‡] FEV₃/FEV₆ ≥ LLN data was used as the reference category.

¹ Models controlled for sex, age, race, body mass index, smoking history (pack-years of smoking) and CT scanner type

² Models controlled for sex, age, race, body mass index and smoking history (pack-years of smoking)

e-Table 2. General linear regression models of difference in quantitative CT indices, quality of life and 6 minute walk distance in participants with FEV₃/FEV₆ <LLN among those with FEV₁/FVC ≥LLN and a negative bronchodilator response (n=3868).

	Gas Trapping% ^{†1}	Wall area% ^{†1}	E/I MLA% ^{†1}	SGRQ ^{†2}	6MWD ^{†2}	mMRC ²	BODE ²
	% difference	% difference	% difference	% difference	% difference	OR	OR
FEV₃/FEV₆ <LLN vs.							
FEV₃/FEV₆ ≥LLN*	28.8	1.7	2.2	37.2	-7.3	1.6	2.9
95% CI	18.3 - 40.3	1.3 - 2.1	1.6 - 2.8	23.5 - 52.5	-9.6 - (-4.8)	1.4 - 1.9	2.1 - 3.9
e^β	1.288	1.017	1.022	1.372	0.927	-	-
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[†] Gas trapping%, E/I MLA, wall area%, SGRQ and 6MWD were natural log transformed. The displayed coefficients (% difference and CI 95%) are back-transformed regression coefficients that correspond to the relative differences between the two groups in percent in ratios. OR indicates the relative odds increase for a higher score of mMRC or BODE between the two groups. * FEV₃/FEV₆ ≥ LLN data was used as the reference category. ¹ Models controlled for sex, age, race, body mass index, smoking history (pack-years of smoking) and CT scanner type. ² Models controlled for sex, age, race, body mass index and smoking history (pack-years of smoking). Positive bronchodilator response was defined as an increase in FEV₁ and/or FVC ≥12% of baseline and 200 ml¹⁵.

Definition of abbreviations: E/I MLA, expiratory to inspiratory mean lung attenuation; SGRQ, St. George Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; OR, odds ratio; CI 95%, exponential (back-transformed) 95% confidence intervals, e^{β} : exponential (back-transformed) regression coefficients.

e-Table 3. Comparison of spirometric, quantitative CT, quality of life, 6 minute walk distance, and clinical variables among subjects stratified by pre-bronchodilator FEV₁/FVC and FEV₃/FEV₆ normalcy.

	Both \geq LLN ¹	Only FEV ₃ /FEV ₆ <LLN ²	Only FEV ₁ /FVC <LLN ³	Both <LLN ⁴	<i>P</i>					
					<i>I</i> vs2	<i>I</i> vs 3	<i>I</i> vs 4	2vs 3	2vs 4	3vs 4
n, (%)	3709 (47.2)	677 (8.6)	179 (2.3)	3288 (41.9)						
Age, years	57 (51 – 64)	59 (52 – 67)	61 (53 – 67)	63 (56 – 69)	NS	*	*	NS	*	NS
BMI	29.6 ± 5.9	28.4 ± 6.3	29.8 ± 6.4	27.7 ± 6.0	*	NS	*	NS	NS	*
Sex, Male %	1964 (53.0)	304 (44.9)	127 (70.9)	1808 (55.0)	NS	NS	NS	NS	NS	NS
Race, Caucasian %	2414 (65.1)	403 (59.5)	147 (82.1)	2610 (79.4)	NS	NS	NS	NS	NS	NS
Duration of smoking, pack.years ⁻¹ (n=7,755)	35 (23 – 48)	38 (28 – 53)	38 (26 – 53)	46 (34 – 66)	*	NS	*	NS	*	*
FEV ₁ %pred	90.4 ± 15.1	79.1 ± 15.6	78.4 ± 15.5	51.3 ± 21.7	*	*	*	NS	*	*
FEV ₃ %pred	96.5 ± 17.2	88.3 ± 18.0	89.7 ± 17.8	66.0 ± 23.1	*	*	*	NS	*	*
FEV ₆ %pred	91.8 ± 14.6	86.7 ± 16.8	87.9 ± 16.2	69.1 ± 20.3	*	NS	*	NS	*	*
FVC %pred	91.8 ± 14.9	87.4 ± 17.1	92.6 ± 18.1	76.8 ± 20.6	*	NS	*	*	*	*
FEV ₁ /FVC, %	76.4 (72.7–79.8)	70.2 (68.2 – 72.1)	65.1 (63.6 – 66.6)	51.5 (38.8 – 61.1)	*	*	*	*	*	*
FEV ₃ / FEV ₆ , %	93.6 (92.6 – 94.7)	90.1 (89.8 – 91.4)	91.9 (91.1– 92.5)	84.7 (78.1 – 88.7)	*	*	*	*	*	*

LAA _{-950insp} , %	0.97 (0.4 – 2.5)	1.03 (0.4 – 2.8)	2.5 (0.8 – 5.9)	7.8 (2.4 – 19.3)	NS	*	*	*	*	*
LAA _{-856exp} , %	8.6 (4.0 – 15.1)	10.8 (5.3 – 19.6)	15.2 (8.3 – 24.7)	35.6 (19.3 – 54.7)	*	*	*	*	*	*
E/I MLA	0.83 (0.79 – 0.87)	0.85 (0.82 – 0.89)	0.85 (0.82 – 0.88)	0.92 (0.88 – 0.95)	*	*	*	NS	*	*
Wall Area, %	60.2 ± 3.0	61.4 ± 3.2	61.1 ± 2.9	62.6 ± 3.1	*	*	*	NS	*	*
SGRQ	10.4 (3.0 – 27.0)	19.9 (6.2 – 38.3)	13.4 (5.8 – 31.1)	37.5 (19.8 – 55.0)	*	NS	*	NS	*	*
mMRC (n=7,849)	0.8 ± 1.2	1.2 ± 1.4	1.0 ± 1.4	2.0 ± 1.4	*	NS	*	NS	*	*
6MWD, meters (n=7,756)	444 ± 108	415 ± 116	451 ± 109	372 ± 122	*	NS	*	*	*	*
BODE (n=7,756)	0.5 ± 0.9	0.9 ± 1.3	0.8 ± 1.3	2.6 ± 2.1	*	NS	*	NS	*	*

Data are mean ± standard deviation, median (IQR 25-75), or median (min, max). All of the comparisons across four groups $P < 0.0001$. Statistically significant in post-hoc comparisons if $P < 0.0083$. NS represents non-significant. * represents $P < 0.0001$. Statistical significance was determined by one-way analysis of variance with Tukey *post-hoc* tests for continuous normal data, Kruskal-Wallis test followed by Mann-Whitney *U* test for pairwise comparisons for continuous non-normal data, and Pearson *chi-square* test for comparison of proportions. Definition of abbreviations: n, number of subjects; BMI, body mass index; FEV₁ %predicted, forced expiratory volume in 1 seconds expressed as percent predicted; FEV₃ %predicted, forced expiratory volume in 3 seconds expressed as percent predicted; FEV₆ %predicted, forced expiratory volume in 6 seconds expressed as percent predicted; FVC %predicted, forced vital capacity expressed as percent predicted; LAA_{-950insp}, total percentage of all lung voxels less than or equal to –950 Hounsfield Units (HU) on end-inspiratory scans, commonly called emphysema%; LAA_{-856exp}, total percentage of all lung voxels less than or equal to –856 Hounsfield Units (HU) on expiratory scans, commonly called gas trapping%; E/I MLA, expiratory to inspiratory ratio of mean lung attenuation; WA, wall area; SGRQ, St. George's Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; LLN, lower limit of normal.

e-Table 4. General linear regression models of the difference in quantitative CT, quality of life, 6 minute walk distance, dyspnea perception and BODE index in models comparing abnormality in FEV₃/FEV₆ and/or FEV₁/FVC in the entire study population (n=7,853).

	Emphysema% ¹	Gas trapping% ¹	Wall Area % ¹	E/I MLA % ¹	SGRQ ²	6MWD ²	mMRC ²	BODE ²
	%difference	% difference	% difference	% difference	% difference	% difference	OR	OR
	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI
Only FEV₃/FEV₆								
<LLN [‡]	11.3†	24.1*	1.8*	2.3*	40.1*	- 6.9*	1.6*	2.5*
(n=677)	(1 – 23)	(16 – 33)	(1.4 – 2.2)	(1.7 – 2.8)	(28 – 53)	(-10 – (-4))	(1.38 – 1.89)	(1.90 – 3.22)
Only FEV₁/FVC								
<LLN [‡]	78.1*	47.5*	1.8*	1.9*	32.4†	-1.1	1.5*	2.6*
(n=179)	(48 – 114)	(30 – 68)	(1.0 – 2.5)	(1.0 – 2.9)	(13 – 55)	(-6 – 4)	(1.10 – 1.97)	(1.63 – 4.16)
Both FEV₃/FEV₆ and FEV₁/FVC[‡]								
<LLN (n=3,288)	350.5*	206.2*	4.5*	8.0*	172.1*	- 18.8*	4.9*	15.2*
	(324 – 378)	(193 – 220)	(4.2 – 4.7)	(7.6 – 8.3)	(158 – 187)	(-20 – (-17))	(4.49 – 5.46)	(12.87 – 17.81)

Emphysema%, gas trapping %, wall area %, E/I MLA, SGRQ and 6MWD were natural log transformed. The displayed coefficients (% difference and CI 95%) are back-transformed regression coefficients that correspond to the relative differences between the two groups in percent in ratios. For example, for emphysema%, the expected mean emphysema% of only FEV₃/FEV₆<LLN group is higher than the both FEV₃/FEV₆

\geq LLN and FEV₁/FVC \geq LLN by 11%. OR indicates the relative odds increase for a higher score of mMRC or BODE between the two groups. For example, the estimated odds of having a one unit higher score of mMRC dyspnea score (worsening from 0 to 4) for only FEV₃/FEV₆ <LLN is 1.61 of the odds compared with both FEV₃/FEV₆ \geq LLN and FEV₁/FVC \geq LLN. ‡ Participants with both FEV₃/FEV₆ \geq LLN and FEV₁/FVC \geq LLN were used as the reference category. * $P < 0.0001$; † $P < 0.05$. ¹ Models controlled for sex, age, race, body mass index, smoking history and CT scanner type. ² Models controlled for sex, age, race, body mass index and smoking history. Definition of abbreviations: FEV₁, forced expiratory volume in 1 second; FEV₃, forced expiratory volume in 3 seconds; FEV₆, forced expiratory volume in 6 seconds; FVC, forced vital capacity; E/I MLA, expiratory to inspiratory mean lung attenuation; SGRQ, St. George's Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walk distance (meters); OR, odds ratio; 95% CI, exponential (back-transformed) 95% confidence intervals. The BODE index includes body mass index (BMI), post-bronchodilator FEV₁% predicted, mMRC score, and the 6MWD.

e-Table 5. Comparison of functional, structural and clinical impairments in FEV₁/FVC ≥LLN female and males divided into subgroups of normal and abnormal FEV₃/FEV₆ (n=4386).

FEMALES, N = 2118			
	FEV ₃ /FEV ₆ ≥ LLN	FEV ₃ /FEV ₆ < LLN	P
	n = 1962	n = 304	
	(%)	(%)	
FEV ₁ %pred < 65	5.0	18.0	< 0.0001
mMRC ≥ 2	28.9	40.2	< 0.0001
SGRQ > 25	29.5	43.2	< 0.0001
6MWD < 350 m	18.7	28.1	< 0.0001
BODE ≥ 2	18.3	27.8	< 0.0001
Gas trapping% ≥ 21	9.5	16.9	< 0.0001
Emphysema% ≥ 3.5	12.3	13.7	NS
MALES, N = 2268			
	FEV ₃ /FEV ₆ ≥ LLN	FEV ₃ /FEV ₆ < LLN	P
	n= 1745	n= 373	
	(%)	(%)	
FEV ₁ %pred < 65	4.2	19.4	< 0.0001
mMRC ≥ 2	20.3	32.2	< 0.0001
SGRQ > 25	24.7	40.8	< 0.0001
6MWD < 350 m	14.9	22.4	0.001
BODE ≥ 2	13.7	25.3	< 0.0001
Gas trapping% ≥ 21	15.4	29.9	< 0.0001
Emphysema% ≥ 3.5	20.5	27.0	0.01

Data are column percentages representing proportion of the population, (lower and upper bounds for 95% confidence interval). Statistical significance was determined by Pearson *chi-square*. Definition of abbreviations: n, number of subjects; FEV₁ %predicted, forced expiratory volume in 1 seconds expressed as percent predicted; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; NS: non-significant.

e-Table 6. Comparison of spirometric, quantitative CT, quality of life, 6 minute walk distance, and clinical variables among subjects stratified by pre-bronchodilator FEV₁/FVC and FEV₁/FEV₆ normalcy.

	Both \geq LLN ¹	Only FEV ₁ /FEV ₆ <LLN ²	Only FEV ₁ /FVC <LLN ³	Both <LLN ⁴	<i>P</i>					
					<i>I</i> vs2	<i>I</i> vs 3	<i>I</i> vs 4	2vs 3	2vs 4	3vs 4
n, (%)	4142 (52.7)	244 (3.1)	283 (3.6)	3184 (40.5)						
Age, years	57 (51 – 64)	59 (52 – 67)	60 (53 – 67)	63 (56 – 69)	†	*	*	NS	*	*
BMI	29.5 ± 5.9	27.9 ± 6.3	29.2 ± 5.5	27.7 ± 6.1	*	NS	*	NS	NS	*
Sex, Male %	52.1	44.7	67.5	54.8	§	*	§	*	†	*
Race, Caucasian %	65.0	51.6	84.8	79.1	*	*	*	*	*	§
Duration of smoking, pack.years ⁻¹ (n=7,755)	35 (23 – 48)	39 (25 – 54)	40 (29 – 58)	46 (34 – 65)	NS	*	*	NS	*	*
FEV ₁ %pred	89.5 ± 15.4	74.8 ± 15.2	80.2 ± 14.3	50.2 ± 21.1	*	§	*	*	*	*
FEV ₆ %pred	91.3 ± 14.9	85.5 ± 17.3	87.1 ± 15.1	68.6 ± 20.2	*	NS	*	*	*	*
FVC %pred	91.5 ± 15.2	84.8 ± 18.9	94.4 ± 17.5	76.2 ± 20.3	*	*	*	*	NS	*
FEV ₁ /FVC, %	75.7 (72.0 – 79.4)	68.3 (67.0 – 70.0)	65.4 (63.7 – 66.7)	50.8 (38.5 – 60.5)	*	*	*	†	*	*
FEV ₁ / FEV ₆ , %	78.7 (75.8 – 81.7)	70.3 (69.5 – 71.5)	73.0 (71.9 – 74.3)	58.0 (47.2 – 66.4)	*	*	*	*	*	*
LAA ₋₉₅₀ insp, %	0.96 (0.4 – 2.4)	1.8 (0.6 – 3.9)	2.0 (0.8 – 3.9)	8.4 (2.6 – 20.1)	*	*	*	NS	*	*

LAA _{-856exp} , %	8.6 (4.0 – 15.1)	16.1 (9.7 – 26.0)	13.0 (7.1 – 20.8)	36.8 (20.6 – 55.5)	*	*	*	NS	*	*
E/I MLA	0.83 (0.79 – 0.87)	0.88 (0.84 – 0.91)	0.84 (0.79 – 0.88)	0.92 (0.88 – 0.96)	*	NS	*	*	*	*
Wall Area, %	60.3 ± 3	61.9 ± 3.5	60.7 ± 2.7	62.7 ± 3.1	*	NS	*	*	†	*
SGRQ	10.9 (3.3 – 28.6)	21.2 (7.7 – 41.5)	12.9 (5.6 – 28.8)	38.4 (21.2 – 55.5)	*	NS	*	†	*	*
mMRC (n=7,849)	0.9 ± 1.2	1.3 ± 1.4	1.0 ± 1.3	2.0 ± 1.4	*	NS	*	NS	*	*
6MWD, meters (n=7,756)	442 ± 108	397 ± 118	452 ± 103	369 ± 121	*	NS	*	*	†	*
BODE (n=7,756)	0.5 ± 1.0	1.1 ± 1.4	0.6 ± 1.0	2.7 ± 1.4	*	NS	*	NS	*	*

Data are mean ± standard deviation, median (IQR 25-75), or median (min, max). * $P < 0.0001$, † $P < 0.01$, § $P < 0.05$. Statistical significance was determined by one-way analysis of variance with Tukey *post-hoc* tests for continuous normal data, Kruskal-Wallis test followed by Mann-Whitney *U* test for pairwise comparisons for continuous non-normal data, and Pearson *chi-square* test for comparison of proportions. Definition of abbreviations: n, number of subjects; BMI, body mass index; FEV₁ %predicted, forced expiratory volume in 1 seconds expressed as percent predicted; FEV₆ %predicted, forced expiratory volume in 6 seconds expressed as percent predicted; FVC %predicted, forced vital capacity expressed as percent predicted; LAA_{-950insp}, total percentage of all lung voxels less than or equal to –950 Hounsfield Units (HU) on end-inspiratory scans; LAA_{-856exp}, total percentage of all lung voxels less than or equal to –856 Hounsfield Units (HU) on expiratory scans; E/I MLA, expiratory to inspiratory ratio of mean lung attenuation; WA, wall area; SGRQ, St. George’s Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walking distance; LLN, lower limit of normal.

e-Table 7. General linear regression models of the difference in quantitative CT, quality of life, 6 minute walk distance, dyspnea perception and BODE index in models comparing abnormality in FEV₁/FEV₆ and/or FEV₁/FVC in the entire study population (n=7,853).

	Emphysema% ¹	Gas trapping% ¹	Wall Area % ¹	E/I MLA % ¹	SGRQ ²	6MWD ²	mMRC ²	BODE ²
	% difference	% difference	% difference	% difference	% difference	% difference	OR	OR
	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI
Only FEV₁/FEV₆								
<LLN [‡]	43.0*	62.5*	2.7*	3.6*	50.3*	- 8.3*	1.7*	2.6*
(n=244)	(22 – 68)	(46 – 81)	(2.1 – 3.3)	(2.8 – 4.4)	(31 – 72)	(-13 – -4)	(1.35 – 2.20)	(1.79 – 3.71)
Only FEV₁/FVC								
<LLN [‡]	42.2*	28.6*	0.9*	0.9†	21.9†	0.0	1.4†	1.2
(n=283)	(23 – 64)	(16 – 42)	(0.4 – 1.5)	(0.2 – 1.7)	(0.7 – 39)	(-0.4 – 0.5)	(1.08 – 1.73)	(0.78 – 1.96)
Both FEV₁/FEV₆								
and FEV₁/FVC[‡]	373.0*	217.1*	4.5*	8.0*	171.1*	- 18.9*	5.0*	14.5*
<LLN (n=3,184)	(346 – 402)	(204 – 231)	(4.2 – 4.7)	(7.7 – 8.3)	(157 – 185)	(-20 – -17)	(4.50 – 5.47)	(12.46 – 16.86)

Emphysema%, gas trapping%, E/I MLA, wall area %, SGRQ and 6MWD were natural log transformed. The displayed coefficients (% difference and CI 95%) are back-transformed regression coefficients that correspond to the relative differences between the two groups in percent in ratios.

* $P < 0.0001$; † $P < 0.05$. ‡ Participants with both FEV₁/FEV₆ \geq LLN and FEV₁/FVC \geq LLN were used as the reference category. ¹ Models

controlled for sex, age, race, body mass index, smoking history and CT scanner type.² Models controlled for sex, age, race, body mass index and smoking history. Definition of abbreviations: FEV₁, forced expiratory volume in 1 second; FEV₆, forced expiratory volume in 6 seconds; FVC, forced vital capacity; E/I MLA, expiratory to inspiratory mean lung attenuation; SGRQ, St. George's Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walk distance (meters); OR, odds ratio; 95% CI, exponential (back-transformed) 95% confidence intervals.

e-Table 8. Univariate correlations (*r*) between spirometric measurements quantitative CT, quality of life, 6 minute walk distance, dyspnea perception and BODE index in the whole study population (n=7,853).

Spirometry	LAA_{950insp} %	LAA_{856exp} %	E/I MLA	Wall Area %	SGRQ	6MWD	mMRC	BODE
FEV₁, %pred	- 0.46	- 0.58	-0.60	- 0.47*	- 0.55	0.46*	- 0.49	- 0.68
FEV₃, %pred	- 0.38	-0.49	-0.51	-0.46*	-0.53	0.44*	-0.47	-0.65
FEV₆, %pred	- 0.32	-0.45	-0.49	-0.45*	-0.50	0.44*	-0.46	-0.62
FVC, % pred	- 0.21	- 0.33	-0.40	- 0.44*	- 0.44	0.42*	- 0.41	- 0.55
FEV₁/FVC, %	- 0.64	- 0.72	-0.65	- 0.31	- 0.44	0.31	- 0.39	- 0.58
FEV₁/FEV₆, %	- 0.63	- 0.73	-0.67	- 0.36	- 0.46	0.33	- 0.41	- 0.60
FEV₃/FEV₆, %	- 0.61	- 0.71	-0.65	- 0.33	- 0.45	0.33	- 0.41	- 0.59
FEV₃/FVC, %	- 0.62	- 0.68	-0.60	- 0.27	- 0.40	0.31	- 0.37	- 0.53
LAA_{950insp}, %	-	0.80	0.48	NS	0.25	- 0.16	0.25	0.38
LAA_{856exp}, %	0.80	-	0.85	0.18	0.34	- 0.30	0.32	0.49
E/I MLA	0.48	0.85	-	0.32	0.37	- 0.36	0.34	0.51
Wall Area %	NS	0.18	0.32	-	0.38	- 0.35*	0.32	0.39

All reported correlations are $P < 0.0001$. The listed values are Spearman correlation coefficients unless stated otherwise. * Pearson correlation coefficients. Definition of abbreviations: $LAA_{-950\text{insp}}$, total percentage of all lung voxels less than or equal to -950 Hounsfield Units (HU) on end-inspiratory scans; $LAA_{-856\text{exp}}$, total percentage of all lung voxels less than or equal to -856 Hounsfield Units (HU) on expiratory scans; E/I MLA, expiratory to inspiratory ratio of mean lung attenuation; SGRQ, St. George's Respiratory Questionnaire; mMRC, modified Medical Research Council Dyspnea Scale; 6MWD, 6 minute walk distance (meters); FEV_1 %pred, forced expiratory volume in 1 second expressed as percent predicted; FEV_3 %pred, forced expiratory volume in 3 seconds expressed as percent predicted; FEV_6 %pred, forced expiratory volume in 6 seconds expressed as percent predicted; FVC %pred, forced vital capacity expressed as percent predicted; NS, non-significant.

Institutional Review Board Committee name and project approval number for 21 study centers.

Clinical Center	Institution Title	Protocol Number
National Jewish Health	National Jewish IRB	HS-1883a
Brigham and Women's Hospital	Partners Human Research Committee	2007-P-000554/2; BWH
Baylor College of Medicine	Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals	H-22209
Michael E. DeBakey VAMC	Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals	H-22202
Columbia University Medical Center	Columbia University Medical Center IRB	IRB-AAAC9324
Duke University Medical Center	The Duke University Health System Institutional Review Board for Clinical Investigations (DUHS IRB)	Pro00004464
Johns Hopkins University	Johns Hopkins Medicine Institutional Review Boards (JHM IRB)	NA_00011524
Los Angeles Biomedical Research Institute	The John F. Wolf, MD Human Subjects Committee of Harbor- UCLA Medical Center	12756-01
Morehouse School of Medicine	Morehouse School of Medicine Institutional Review Board	07-1029
Temple University	Temple University Office for Human Subjects Protections Institutional Review Board	11369

University of Alabama at Birmingham	The University of Alabama at Birmingham Institutional Review Board for Human Use	FO70712014
University of California, San Diego	University of California, San Diego Human Research Protections Program	070876
University of Iowa	The University of Iowa Human Subjects Office	200710717
Ann Arbor VA	VA Ann Arbor Healthcare System IRB	PCC 2008-110732
University of Minnesota	University of Minnesota Research Subjects' Protection Programs (RSPP)	0801M24949
University of Pittsburgh	University of Pittsburgh Institutional Review Board	PRO07120059
University of Texas Health Sciences Center at San Antonio	UT Health Science Center San Antonio Institutional Review Board	HSC20070644H
Health Partners Research Foundation	Health Partners Research Foundation Institutional Review Board	07-127
University of Michigan	Medical School Institutional Review Board (IRBMED)	HUM00014973
Minneapolis VA Medical Center	Minneapolis VAMC IRB	4128-A
Fallon Clinic	Institutional Review Board/Research Review Committee Saint Vincent Hospital – Fallon Clinic – Fallon Community Health Plan	1143