STUDY PROTOCOL: GENETIC EPIDEMIOLOGY OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPDGene®)

I. BACKGROUND AND SIGNIFICANCE

Chronic obstructive pulmonary disease (COPD) is the third leading cause of mortality in the United States (1). COPD is a heterogeneous syndrome, with varying contributions of emphysema and airway disease in each COPD subject. Genetic studies of complex diseases like COPD have the potential to provide insight into the pathophysiological mechanisms of COPD susceptibility and heterogeneity. A strong genetic basis for the susceptibility of smokers to develop COPD is suggested by:

(1) Marked variability in the development of airflow obstruction among smokers (2)

(2) Clear familial clustering of COPD and COPD-related phenotypes (3) and

(3) Evidence of linkage for COPD-related phenotypes to specific genomic regions in families with severe, early-onset COPD (4).

COPD is a heterogeneous condition with variation in rates of disease progression, symptoms and CT characteristics. We have previously identified CT subtypes of disease based on visual scoring and found variation in progression of disease based on spirometry and CT metrics (emphysema, airway disease).

Case-control studies have been performed for many candidate genes in COPD, but the results have been inconsistent (5). Recent progress in single nucleotide polymorphism (SNP) genotyping and DNA sequencing allows for association studies on a genome-wide scale; however, the multiple statistical tests involved in genome-wide association (GWA) studies of hundreds of thousands of SNPs raise challenges in separating true from false positive associations (6). In addition, genetic association studies within a single racial/ethnic group may not generalize to other populations.

To address the multiple testing and generalizability problems of GWA studies, we propose to perform a comprehensive GWA and DNA sequencing study to identify genes influencing COPD in two major racial/ethnic groups (non-Hispanic Whites and African Americans). Our primary hypotheses are:

(1) Precise characterization of COPD subjects using computed tomography – as well as clinical and physiological measures assessed longitudinally – will provide insight that will enable the broad COPD syndrome to be decomposed into clinically significant subtypes.

(2) Genome-wide association and DNA sequencing studies will identify genetic determinants for COPD susceptibility that will provide insight into clinically relevant COPD subtypes.

(3) Distinct genetic determinants influence the development of emphysema and airway disease.

COPDGene Phase 3

COPDGene has been funded for an additional five years by the US National Heart, Lung, and Blood Institute as well as the COPD Foundation. In addition to the cross-sectional subject data collected in the baseline and five year follow up COPDGene study visits, disease progression and incidence of COPD in smokers are important additional endpoints for genetic association studies. In Phase 1, we have completed enrollment of 10,371 subjects. In Phase 2, we have completed 5,835 return visits and an additional 574 other follow up methods. For Phase 3, we now propose to invite these subjects to return for a third evaluation ten years after their initial visit to assess disease incidence and/or progression in COPDGene subjects.

II. SPECIFIC AIMS

The current specific aims for Phase 3 of COPDGene are included in this section.

Specific Aim 1: Disease Progression

All subjects who return for a 10-year visit and those who expire between the 5 and 10-year visits will be analyzed to confirm prognostication of clinical disease progression. We estimate that 6,500 subjects will be analyzed in this aim; 4,500 subjects who return for a 10-year visit and 2,000 who expire before that visit.

Specific Aim 2: Mortality and Incident Disease

All 10,371 subjects enrolled in Phase 1 will be included to identify genetic, transcriptomic, and proteomic determinants of susceptibility and progression of specific COPD subtypes. All these subjects have had an initial visit but may or may not have visits at 5 and 10 years.
Through the longitudinal follow-up (LFU) program conducted twice a year since the initial visit, we have obtained follow-up data from 82.3% of the original 10,371 subject cohort by a combination of telephony, web survey or coordinator calls. We will continue LFU on all of the original 10,371 subjects who are alive. All subjects will continue to be followed to assess vital status. We will perform death searches at least yearly to identify subjects that have expired. Death certificates, medical records and informant interviews will be obtained to determine cause of death.

Specific Aim 3: Rapid Disease Progression Subtypes (Short Term Follow-up Study)

To test high-risk subtypes for rapid COPD progression, approximately 800 subjects from the full COPDGene cohort will participate. These subjects will be invited back for a Short Term Follow up Study visit approximately one year after the Phase 3 visit.
Subjects participating in the Short-Term Follow-Up study will be identified by the Data Coordinating Center and a list will be provided to each participating clinical center.

III. SUBJECT SELECTION- Phase 3

In summary, Phase 3 is the 10-year and 11-year (short-term follow up for applicable subjects) return visit. Phase 3 is also the 5-year return visit for Non-Smokers enrolled in Phase 2.

All subjects to be studied have been enrolled in the Genetic Epidemiology of COPD Study (COPDGene) in previous phases. As part of their original informed consent for COPDGene and subsequent consent at the 5-year follow-up visit, subjects have provided consent to be re-contacted for periodic follow-up and for other studies.

This project will invite all 10,371 subjects who are alive and still in the study who successfully completed a Phase 1 visit for a Phase 3 study visit. At the time of writing this protocol, there are 1,573 deceased subjects. All non-smokers enrolled in Phase 2 will also be eligible to return for a visit 5 years (± 6 month) after their first visit, in total 349 new non-smokers were enrolled in Phase 2. This is a total of 10,720 subjects in the cohort. Phase 3 will be scheduled 10 years (± 6 months) after Phase 1 or 5 years (± 6 month) after Phase 2 (for new subjects enrolled in Phase 2). There must be at least 3 years in between the Phase 2 and Phase 3 visits. Visits later than the scheduled return visit *are* permitted. The Short-Term Follow-Up Study for rapid progressors will occur approximately one year after the subject participates in the Phase 3 study.

A key component of the project is to establish contact with all of the previously enrolled subjects who remain in the study or confirm the subjects are deceased. Clinical centers will utilize locally stored contact information including secondary contacts to establish contact.

Eligible subjects will be contacted mail, email and/or phone and invited to participate in Phase 3 at the Clinical Centers. The study protocol visit will be discussed in detail during an initial screening encounter. Subjects will also be asked pre-screening questions to determine if they are eligible to return at that time. All subjects who participated in Phase 1-Visit 1 are eligible to return for Phase 3; however, there are some temporary visit exclusions, which are described later in this section. Before the study visit or any study-related procedures begin, written or verbal informed consent will be obtained by one of the research study staff members. A physician investigator from the Clinical Center will be available to answer any questions during the informed consent process.

A summary of the visit procedures previously conducted in Phase 1 and Phase 2 are attached to this protocol as Appendix A (Phase 1) and Appendix B (Phase 2).

Subject Inclusion/Exclusion Criteria – Phase 3 Return Visit

Inclusion Criteria

Previously enrolled in COPDGene and meeting initial eligibility criteria at Visit 1.

Temporary Exclusion Criteria

Subjects that meet the following will be temporarily excluded but may be rescheduled at a later time:

1. Pregnancy or suspected pregnancy. (These subjects will be re-scheduled for a visit at least three months after childbirth or after they are no longer suspected of pregnancy.)
2. Use of antibiotics for a respiratory infection within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the antibiotics.)
3. Use of systemic corticosteroids (new prescription or increased dose) for a respiratory disorder within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the steroids.)
4. Chest or abdominal surgery in the past three months. (These subjects will be rescheduled for a visit at least three months after surgery.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)
5. Heart attack in the last three months. (These subjects will be rescheduled for a visit at least three months after the heart attack.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)
6. Detached retina or eye surgery in the past three months. (These subjects will be re-scheduled for a visit at least three months after surgery.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)
7. Hospitalization for any other heart problem in the past month. (These subjects will be rescheduled for a visit at least 4 weeks after hospital discharge.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)

Subjects with lung transplants will be excluded from having a follow-up chest CT scan; however, they will be invited back to complete all other portions of the visit.

Rationale: All previously enrolled subjects from COPDGene are eligible for the study. Previous exclusions for lung cancer and other conditions are not a reason to exclude subjects from follow-up evaluation because these are outcomes to be assessed.

We will temporarily exclude patients with recent use of antibiotics and/or systemic steroids for a COPD exacerbation since such subjects may have an ongoing inflammatory pulmonary process related to an infection that will impair our ability to characterize and phenotype patients in their usual stable state.

We will temporarily exclude subjects who have contraindications to spirometry (exclusion criteria 4 – 7 above).

Subjects with temporary exclusion criteria will be re-screened at a later date when they can be enrolled in Phase 3. The Administrative Core should be contacted regarding subjects who cannot complete all components of the study visit due to temporary exclusions or other medical conditions. The local Clinical Center Director will determine which portions of a study visit can be completed. Those modifications will be considered if the subject is unlikely to be able to return at a later date for the study visit or unlikely to resolve medical issues that limit participation.

New Non-Smoker Controls

To reach our Phase 1 goal of up to 12,000 subjects, we plan to enroll up to an additional 1,000 subjects in Phase 3, if funding is obtained. We will target enrollment of additional non-smokers without lung disease. By providing insight into the normal effects of aging in healthy subjects, these subjects will allow us to correctly interpret the chest CT scans, lung function, health status and other features of our current cohort. For example, aging is associated with loss of lung elasticity, a characteristic feature of emphysema. Without knowledge of the effects of age, gender and race on the lungs of healthy subjects, we cannot accurately determine whether CT scans of subjects with a smoking history are normal or abnormal, or assess disease severity.

Subject Inclusion/Exclusion Criteria for <u>Non-smoker Controls</u>

<u>Inclusion Criteria</u>

- Age 45-85 years
- Non-Hispanic Whites and African Americans
- No smoking history as defined by less than 100 cigarettes smoked in a lifetime, less than 52 cigars smoked in a lifetime, and less than 12 oz. of pipe tobacco smoked in a lifetime

<u>Exclusion Criteria</u>

- Respiratory disorders (including, but not limited to, COPD, asthma, bronchiectasis, cystic fibrosis, or interstitial lung disease)
- Any lung surgery
- Lung cancer
- History of Pneumothorax
- Pleural procedure (including, but not limited to, chest tube placement, pleural surgery)
- History of any pulmonary procedures: diagnostic bronchoscopy, lung biopsy, except research bronchoscopy
- Uncontrolled cancer, as defined as ongoing radiation therapy, ongoing chemotherapy, narcotics for pain control, or known metastatic disease
- History of radiation therapy to the chest (other than radiation for breast cancer)
- Subjects receiving treatment for active TB
- Severe cirrhosis
- Renal disease requiring dialysis
- Pulmonary Hypertension
- Congestive heart failure
- Inability to use albuterol
- First or second- degree relative (parent, brother, sister, daughter, son, aunt, uncle, nephew, niece, half-sibling, grandparent, grandchild) of a subject enrolled in COPDGene
- Subjects who indicate they are in more than one racial category
- Subjects who do not authorize the storage of personal identifiers (social security number, name, address, phone number) in the COPDGene Data Coordinating Center
- Metal objects that may interfere with chest CT quantification including presence of a cardiac pacemaker, defibrillator, metal prosthetic heart valve, metal projectile or metal weapon fragment (bullet, shrapnel, shotgun shot) or metal shoulder prosthesis
- Participation in the following studies: COPDGene Phase 1, ECLIPSE, Boston Early-Onset COPD Study, Boston COPD Exacerbations Study, Denver Genetics Study, GlaxoSmithKline International COPD Genetics Network, NCI: National Lung Screening Trial, NIH: COPD Clinical Research Network: Macrolide and Leukotriene Trials, NIH: Long-Term Oxygen Therapy Trial, NIH: Lung Health Study, NIH: Lung

Tissue Research Consortium, NIH: SPIROMICS, Pittsburgh SCCOR, MESA, MESA-Lung and CARDIA

Inability to provide personal telephone contact number(s) and complete information for two additional contacts (next of kin, relative or close friend, not living with subject)

No place of permanent residence for the prior three months

Alpha-1 Antitrypsin deficiency

Regular use of an inhaled medication

Regular marijuana use (more than once per week)

HIV Positive

History of transplant of any organ

Active drug or alcohol dependence

History of employment in underground mining

Body mass index greater than 35

Temporary Exclusion Criteria

Subjects that meet the following may not have a study visit performed but may be re-screened at a later time.

1. Pregnancy or suspected pregnancy. (These subjects will be re-scheduled for a visit at least three months after childbirth or after they are no longer suspected of pregnancy.)
2. Use of antibiotics for a respiratory infection within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the antibiotics.)
3. Use of systemic corticosteroids (new prescription or increased dose) for a respiratory disorder within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the steroids.)
4. Chest or abdominal surgery in the past three months. (These subjects will be rescheduled for a visit at least three months after surgery.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)
5. Heart attack in the last three months. (These subjects will be rescheduled for a visit at least three months after the heart attack.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)
6. Detached retina or eye surgery in the past three months. (These subjects will be re-scheduled for a visit at least three months after surgery.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)
7. Hospitalization for any other heart problem in the past month. (These subjects will be rescheduled for a visit at least 4 weeks after hospital discharge.) (Does not apply to screening exclusions for the Phase 3 virtual visit.)

IV. SUBJECT ENROLLMENT- Phase 3

The below table summarizes each phase of COPDGene.

Phase 1- COMPLETED	***Phase 2- COMPLETED***	**Phase 3a- current project**	**Phase 3b- Short Term Follow Up -current project**	**Phase 3 Modified COVID-19**
Visit 1 for all subjects *n=10,371 enrolled*	*Phase 1 subjects followed up in Phase 2* *n=6,409*	Will invite all subjects alive and still in the study from Phase 1 back for Phase 3	Select group of up to 800 subjects characterized as *rapid progressors* from analysis of the Phase 2 data will be invited for a Short –Term Follow-Up study to occur approximately 1 year after the subject is due/returns for their Phase 3 (Phase 3a) study visit.	Will invite all subjects alive, still in the study from Phase 1, and have not yet completed a Phase 3 visit for a virtual visit over the phone to answer some of the questionnaires. Later, those subjects will be brought on-site to complete in-person portion of the visit. All subjects will be invited to provide updated information and medical records through a new questionnaire about respiratory illnesses

	New Non-smoking Subjects enrolled *n=349*	All 349 nonsmokers enrolled in Phase 2 will be invited back for Visit 2 (5 year follow up)		and COVID associated illnesses or abnormal test results.
		New Nonsmokers will be recruited		

All subjects previously enrolled in Phase 1 have provided consent with their initial study enrollment to be contacted again for further research studies. Subjects will be sent an invitation letter advising them that funding has been provided for the Phase 3 visit and informing them that they will be contacted to schedule an appointment. Information learned from previous phases of COPDGene will be included in this initial contact.

A telephone call will be made to each subject to schedule a date for his/her next study visit and ask them to bring the following to the study visit:

1) all of their current oral and inhaled medications,
2) names of any injectable study medications received on a regular basis,
3) social security number,
4) name, address, and phone number of their personal physician,
5) name, address, and phone of two other individuals likely to know the subject's whereabouts, at least one of whom is a relative not living with the subject, and
6) driver's license or other identification to assure they are the individual previously enrolled.

For the virtual phone visit, a phone call will be made to each subject to schedule a time when they can complete the questionnaires over the phone. During this scheduling call, the participant will be asked to assemble their contact information (email, other phone numbers) if they need to look it up.

The pre-eligibility questions will be asked upon scheduling to confirm that the subject is eligible for a visit at that time.

At the Phase 3 study visit, study staff will review the informed consent form and study procedures with the subject. For the modified visit due to COVID-19, the coordinator will read a consent script to the participant before beginning to administer the questionnaires. At the in-person portion of the modified visit, the study staff will review the informed consent form as in a regular visit. The subjects will have the opportunity to review the study documents in private, have questions answered and sign the informed consent. If necessary, subjects may review the informed consent with family members or their physicians. A separate HIPAA or combined consent and HIPAA will be reviewed and signed at the same time. Although subjects have signed a HIPAA in Phase 1 and Phase 2, a current authorization will be obtained to meet the

requirements of hospitals and health care providers. Phase 3 data collection includes transfer of subject identifiers to the Data Coordinating Center (DCC).

Final eligibility will be confirmed prior to conducting the study visit.

V. PHASE 3 STUDY PROCEDURES

Similar to previous COPDGene visits, for the Phase 3 study there will be one to two visits for the majority of subjects depending on whether a separate visit is needed to schedule a chest CT scan or other study procedures. In some cases, subjects may have a third visit to re-collect information that does not meet quality control criteria. For example, if spirometry does not meet quality control criteria as judged by the Pulmonary Function Core, the subject may be contacted by phone to schedule an additional visit to repeat the test. The below table describes all procedures for the Phase 3 Return Subjects and Phase 3 New Nonsmokers, as well as the Phase 3 Short Term Follow Up visits and the Phase 3 modified visit due to COVID-19.

Non-smoker controls enrolled during the Phase 3 study will be assessed for eligibility using the inclusion and exclusion criteria listed previously (Section III) for the new non-smoker controls.

Table of Visit Procedures

Procedure **forms are different for new nonsmokers*	**Phase 3a Visit- Return Subjects and New Nonsmokers**	**Phase 3b- Short Term Follow Up or post-COVID Follow-up**	**Phase 3 Modified- Virtual**	**Phase 3 Modified- In-Person**
Eligibility*	X	X	X	X
Informed Consent/HIPAA	X	X	X	X
Confirmation of Final Eligibility	X	X	X	X
Medical Record Release Authorization	X	X		X
Subject Personal Information	X	X	X	
Demographics Form	X	X		X
Safety Assessment	X	X		X
Medical History*	X	X	X	
Medication History	X	X		X
Pre- and post-bronchodilator spirometry with albuterol	X	X		X

Procedure **forms are different for new nonsmokers*	**Phase 3a Visit-Return Subjects and New Nonsmokers**	**Phase 3b-Short Term Follow Up or post-COVID Follow-up**	**Phase 3 Modified-Virtual**	**Phase 3 Modified-In-Person**
DLco	X	X		X
Respiratory Disease Questionnaire*	X	X	X	
Modified Respiratory Disease Questionnaire				X
Six Minute Walk Test	X	X		X
CAPTURE Questionnaire	X		X	
SF- 36 Questionnaire	X	X	X	
St. George's Respiratory Questionnaire (SGRQ)	X	X	X	
COPD Assessment Test (CAT)	X	X	X	
HADS	X	X		X
Cognitive Screen Items	X			X
Socioeconomic Questionnaire*	X		X	
PROactive Physical Activity Questionnaire	X	X	X	
Duke Activity Status Index (DASI)	X	X	X	
eCigarette Questionnaire	X	X	X	
Cannabis Questionnaire	X		X	
Optimism Questionnaire	X		X	
Residential and Occupational History*	X		X	
PTOA	X		X	
Blood Sample Collection	X	X		X

Procedure **forms are different for new nonsmokers*	Phase 3a Visit-Return Subjects and New Nonsmokers	Phase 3b-Short Term Follow Up or post-COVID Follow-up	Phase 3 Modified-Virtual	Phase 3 Modified-In-Person
Sit to Stand Test	X	X		X
Hand Grip Strength	X	X		X
Food Frequency Questionnaire	X			X
CT Scan	X	X		X
COVID Questionnaire			X	
Dried Blood Spot			X	

During the Phase 3 research study visit, the following procedures will be performed (for return subjects and new non-smokers):

1. Informed consent and HIPAA authorization will be obtained prior to any other study procedure. An updated authorization to obtain medical records to adjudicate the cause of death will be obtained. Subjects will be asked to provide a signed release of medical records to be used in the event of their death, to obtain an interview with a next of kin, secondary contact, or personal representative and with the treating physician. Hospital and physician records, related to the events and illnesses associated with a participant's death, will also be obtained through the signed release of medical records. Subjects will be provided a memo to give to their physician(s) for this purpose.

    a. Medical records related to respiratory illnesses or COVID-19 associated illnesses/abnormal test results will be requested from the subjects. Subjects will be asked to sign a medical record release or obtain records directly and provide the study with copies of those records. Records may also be obtained through Electronic Medical Records (EMR). Records that are requested will include, but not be limited to, emergency room, in-patient or out-patient hospitalization, urgent care, and physician office records as well as imaging studies.

2. Subjects will be asked to provide or update contact information of two secondary contacts with addresses and phone numbers, one of whom is a next of kin or personal representative, and the other a relative or close friend, not living with the subject. The purpose of this information is to locate subjects who have moved to a different home and have a different address and/or phone number and ascertain vital status of the subject. The subjects will be asked to give consent to have their participation in the COPDGene study disclosed to these secondary contacts in the event that we are unable to contact them, and also disclosed to their next of kin, personal representative or family members in the event of their death. Subjects will be provided a memo to give to their secondary contacts for this purpose.

3. Prior to any other evaluations, a final eligibility screening form will be administered. A Safety Assessment will be completed to determine if potential subjects have a condition

that would require deferring their study visit. They will be asked if they have a history of adverse events with the use of albuterol prior to administration of this inhaled bronchodilator. This safety assessment will also be used to ascertain potential cardiac disorders prior to albuterol administration, the walk test and the sit to stand test. If the questionnaire uncovers known or potential adverse effects of albuterol or potential cardiac disorders, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a six-minute walk test and/or sit to stand test.

4. Updated contact information will be collected from the subjects. Name, home address (mailing address and physical address if different), home phone number, cell phone, email address, date of birth and social security number will be collected or verified from the subject. The purpose of collecting this information is to maintain contact with the subjects twice a year and inform the subjects of other research studies and potential future COPDGene follow-up visits pending future funding, and to identify impacts of pollution and other location factors on disease severity and progression. In order to perform central collection of mortality and cause of death, this information will be securely transmitted to the Data Coordinating Center at National Jewish Health, where it will be stored separately from the clinical, physiological, imaging, and genetic data. The transmittal of subjects' personal information to the DCC will be used for central subject tracking, longitudinal follow-up, death searching through the Social Security Death Master File (SSDMF) and/or National Death Index (NDI), and to obtain cost and utilization information from the Centers for Medicare and Medicaid Services (CMS).

5. Physical Assessment will be performed on all subjects: A limited assessment will be performed including height, weight, arm span, waist circumference, blood pressure, heart rate, and room air oxygen saturation by pulse oximetry with the subject seated at rest. Blood pressure will be measured three times with the subject seated and resting for at least five minutes. In subjects using supplemental oxygen, the oxygen will be withheld, and subjects will breathe room air for 10 minutes prior to *recording* oxygen saturation. If resting oxygen saturation falls below 82% or the subject becomes dyspneic prior to 10 minutes without oxygen, oxygen therapy will be restarted as a safety precaution. If heart rate is less than 50 or greater than 120 or if the blood pressure is greater than 170/110, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform the walk test and sit to stand test. Approval to defer or continue will be documented on the Safety Assessment form.

6. Pre- and post-bronchodilator spirometry will be performed on all subjects. The maneuvers are performed according to American Thoracic Society standards. To obtain 3 acceptable measures, the technician may ask the subject to perform up to 8 attempts. Spirometry is performed before and then repeated approximately twenty minutes following the administration of two puffs (180 mcg) of albuterol from a metered dose inhaler with a spacer. Spirometry will ideally be performed with the subject in a seated position with a nose clip in place. These results will be provided to the subject, subjects will be asked to consent if they would like a copy of their test result sent to their primary care provider.

7. Diffusing capacity will be measured on all subjects after post-bronchodilator spirometry. The test will be performed according to American Thoracic Society/European Respiratory Society standards (8). In subjects using supplemental oxygen, oxygen will be removed, and subjects will breathe room air for 10 minutes prior to the test. If resting saturation falls below 82% or the subject becomes dyspneic prior to 10 minutes without oxygen, oxygen

therapy will be restarted as a safety precaution and the test will be performed without the ten-minute oxygen washout. To obtain 2 reproducible measures, the test may be repeated up to 5 times.

8. Standardized questionnaires will be completed by all subjects to assess respiratory symptoms, smoking history, and other medical history. These questionnaires include the St. George's Respiratory Questionnaire to assess health-related quality of life, the Medical Outcomes SF-36 to assess generic quality of life, a COPD screening questionnaire (CAPTURE) (32), the COPD Assessment Test (CAT) to assess COPD severity, and the PROactive (33) and Duke Activity Status Index (DASI) (34) questionnaires to assess physical activity. Questionnaires will be administered to obtain information about respiratory history, medical history, anxiety and depression (Hospital Anxiety and Depression Scale- HADS) (9). If data from the HADS are felt to be clinically significant, they will be sent to the subject's primary care provider. Questions will be asked about menstrual & post-menopausal history in women, oxygen use, and current/previous residential and work history (Residential/Occupational Questionnaire) to ascertain potential air pollution and environmental exposures. Subjects will be asked about access to regular medical care, access to health insurance and attitudes about medical care for their lung disease (Socioeconomic Questionnaire) to determine the impact of these factors on lung disease and exacerbations. An e-Cigarette and a cannabis use questionnaire will also be administered to assess amount of e-Cigarette/vaping/cannabis use and attitudes. Some cognitive testing questions will also be asked of the subject and the Mini-Cog test (31) will be administered. The Mini-Cog includes a 3-word recognition test and a clock drawing. The Mini-Cog clock drawing will be labeled with the subject ID and uploaded to the Administrative Core for adjudication review of the coordinator scoring. Optimism has been associated with longevity and cardiovascular health; prior studies have suggested that optimism is associated with slowed lung function decline and decreased mortality from chronic respiratory disease, even after adjustment for lifestyle choices and cigarette smoking (35). There is little understanding of the biological mechanisms that may drive the association between optimism and respiratory outcomes. The addition of the Optimism Questionnaire adds six questions to the COPDGene Phase 3 study protocol will allow us to study both the epidemiologic and biologic associations between optimism and outcomes in smokers with and without COPD. The Post-Traumatic Osteoarthritis questionnaire asks patients questions about knee arthritis. The additional Pandemic Questionnaire is directed at assessing the impact of COVID-19 on disease progression and also on the overall impact of the pandemic on the subjects with chronic disease and smoking exposure.

Each patient will be asked to bring in all of their current medications in their original bottles. A list of current medications will be made at the local clinical center. Information collected transmitted to the Data Coordinating Center with the subject's study ID number.

A clinical coordinator will administer the questionnaires. The results will be entered directly into the computer or recorded on paper forms to be input later. Subjects may also have the option to complete some of the questionnaires on a computer or tablet. Completion of all questionnaires is expected to take between 90 and 120 minutes.

If a participant is found to have cancer, determined by the Medical History Questionnaire, the subject may be contacted to provide medical records to aid the site in completing a Cancer Data Collection Questionnaire to collect additional data on the cancer diagnosis. In the future, the subject may be invited to sign a tissue release addendum in order to

provide formalin fixed paraffin embedded (FFPE) tissue blocks if there was a cancer diagnosis or lung tissue removed.

If a participant is found to have had any illness that was diagnosed or suspected to be due to COVID-19, or any similar respiratory illness, the subject may be contacted to provide medical records concerning that illness, the subject may sign a medical records release form, or the records may be obtained in EMR.

9. After the visit, each subject will be given a Food Frequency Questionnaire (FFQ) to complete at their convenience. A growing body of literature has linked diet with risk for COPD in smokers (37). However, few studies have addressed the association of additional COPD-related phenotypes such as emphysema in relation to diet. The Food Frequency Questionnaire (FFQ) has been extensively validated (38, 39, 40) in large cohort studies and will provide new information about links between diet and COPD progression, severity, and subtypes. The FFQ is 4 pages long. Each participant will receive the questionnaire, questionnaire instructions, a stamped envelope addressed to the Channing Division of Network Medicine at Brigham and Women's Hospital, and a #2 pencil in a postage paid addressed envelope. The subject has the option to complete the FFQ at the end of the visit or to complete it at home and send it back in the mail. The instructions for the FFQ will be provided on a cover letter that will instruct the subject on proper completion of the questionnaire and also tell them not to write their name or any other identifying information on the questionnaire or return envelope. Each questionnaire will have the subject ID already listed on it before being provided.

10. Blood will be drawn from all subjects for DNA (genetic and epigenetic studies), serum and plasma (for measurement of other proteins potentially related to COPD and other diseases) and a CBC (complete blood count). A total volume of up to 50 ml of blood will be drawn for this study. A complete blood count will be performed at the Clinical Centers. The rest of the blood samples will be stored at the COPDGene Biological Repository at Brigham and Women's Hospital. Blood count results will be sent to the subject. If results from the blood counts are felt to be clinically significant, they will also be sent to the subject's primary care provider. Subjects without clinically significant results will be asked to consent if they would like their blood count results sent to their primary care physician. We will ask the subject what time of day they last ate and/or drink anything other than water; including candy/chewing gum, and collect this information.

    A dried blood spot kit will be sent to the participants who have agreed to provide a blood sample. The kit will include an alcohol swab, lancets, sample collection card, card sleeve, band-aid, instructions, return envelope, and a copy of the informed consent.

    Subsequent blood spot kits will be sent to participants who have had an inconclusive result on their previous antibody test(s) or lost/damaged the kit. Blood spot kit(s) will be sent to participants to survey for COVID antibodies at up to 12-month intervals when COVID remains in an epidemic status and extend into antibody surveys at up to 12-month intervals during the first five years of endemic circulation of COVID.

11. Six-Minute Walk Test will be performed in a standardized manner on all subjects to determine exercise capacity (11). Subjects will be asked questions to assure they do not have significant or occult heart disease prior to the test to assure subject safety. Subjects who have significant cardiovascular disease may require evaluation by the local physician

investigator prior to six-minute walk test. Immediately after and one minute after the end of the six-minute walk test, a subject's heart rate and oxygen saturation will be measured using a pulse oximeter. Subjects who use > 6 L/min oxygen flow with activity will not perform the Six- Minute Walk Test.

12. Chest CT Scans will be performed to assess emphysema and airway disease in all subjects. An inspiratory chest CT scan will be performed using a reduced dose protocol that utilizes dose modulation. Dose modulation automatically adjusts x-ray exposure for each patient to achieve a consistent level of image quality. The reference mAs for this scan will be set such that an average sized subject (~75 kg) with a normal build would receive an x-ray exposure (CTDIvol) of 3.0 mGy (corresponding roughly to an effective dose of 1.5mSv). X-ray exposure will automatically be adjusted higher or lower for larger or smaller subjects. The maximum exposure for the dose modulation acquisition will be set at 3.0 mSv. The scans will be processed using filtered back projection and (where feasible) iterative reconstruction. An expiratory CT acquisition will be performed using a fixed x-ray tube current of 50 mAs to assess for air trapping.

    Results of the CT scan will be provided to the subject. Subjects will be asked to consent if they would like their results sent to their primary care physician. If any clinically significant findings are found on the CT scan, they will be automatically sent to the primary care provider.

    If the chest CT scans are done on the same day as the post-bronchodilator spirometry, the scan should be scheduled as soon as possible after albuterol. The time and date of the last bronchodilator medication use prior to the CT scan will be captured. CT scans will be electronically transmitted to the Imaging Core at National Jewish Health by secure FTP site.

13. The 30 second sit to stand test will be performed. The purpose is to test leg strength and endurance. Blood pressure and heart rate will be checked prior to performing the test. Contraindications would be patients at high fall risk specifically amputees without prosthesis, individuals who need an assistive device for ambulation, subjects with a short term lower extremity impairment, and patients who have had previous stroke with significant residual deficits in balance or proprioception. The subject will sit in a chair with hands placed on the opposite shoulders and crossed at the wrists, then will rise to a full standing position and then sit back down again, repeating the maneuver as many times as possible within 30 sections. The number of times the subject comes to a full standing position is recorded.

14. In ever-smokers with COPD, hand grip strength is associated with computed tomography markers of body composition and airway thickness, independent of body mass index and emphysema. Higher hand grip strength is associated with lower exacerbation frequency (36). Functional testing for hand grip strength measurements will be done using a Jamar dynamometer. Subjects will be asked to squeeze the device with their dominant hand and repeat the maneuver for a total of three measurements.

15. A urine pregnancy test will be performed on all pre-menopausal women prior to the CT scan to confirm that they are not pregnant.

16. Medical Record Review: medical records may be reviewed when available and with a signed release of records form documenting the subject's permission to obtain information

about lung diseases, cancer, other medical conditions, and to determine cause of death, in the event of death.

17. If during the LFU call the participant answers that they had a COVID-19 diagnosis or illness, the coordinator will call the participant to consent for and administer the short-version COVID Questionnaire. If the phone consent is not possible, the coordinator will send the LFU contact letter and the participant will call the coordinator to administer the short-version COVID Questionnaire.

Home Visit

Previously enrolled COPDGene subjects who are unable to come to a COPDGene Clinical Center to complete the Phase 3 visit due to illness, disease progression, or inability to be transported to the Clinical Center, will be given the option of having a research coordinator complete a partial study visit at the subject's place of residence.

If a COPDGene home visit is performed, it will include Home Visit Informed Consent and HIPAA administration, pre and post-bronchodilator spirometry, blood draw, hand-grip strength, sit-to-stand test, and completion of all study questionnaires and procedures, *except* the six-minute walk, diffusing capacity, and CT scan.

VI. OTHER PHASE 3 STUDY PROCEDURES

COVID-19 Adaptation

During the COVID-19 pandemic, COPDGene will allow study sites to stop in-person visits to protect both the subjects and staff. During this time, increased contact with the subject will occur to track COVID-19-related illness. This will be tracked using a brief questionnaire that the subject can choose to answer over the phone with a coordinator, through email, or if the site is allowing in-person visits to occur, then the subject can answer the questionnaire at the time of the visit. The participant will be mailed a dried blood spot kit that will allow them to prick their finger for a blood sample and send that sample back to determine if they have formed antibodies to COVID-19; if the participant is on site for their visit at the time the finger-prick is due, then they may opt for the coordinator or a nurse to assist. Subsequent blood spot kits will be sent to participants who have had an inconclusive result on their previous antibody test(s). Blood spot kit(s) will be sent to participants to survey for covid antibodies at up to 12-month intervals when COVID remains in an epidemic status and extend into antibody surveys at up to 12-month intervals during the first five years of endemic circulation of COVID.

The subject will consent to this increased contact, the collection of blood, and COVID-19 medical records release at the time of consent for the modified visits for the Phase 3 Virtual visit and/ or (for those not scheduled for a phase 3 visit) a separate contact for COVID-19 tracking survey. Type of consent for the COVID-19 portion of Phase 3 may be obtained using a written consent in person or through mail, or a verbal consent over the phone. The Phase 3b visits will be modified to include subjects who experienced a respiratory exacerbation reported on the patient's surveys will be included in the 3b visits as a group potentially at risk for rapid progression. These

subjects will be identified by the DCC and the centers will be asked to invite the subjects to return for a phase 3b visit.

If during the LFU call the participant answers that they had a COVID-19 diagnosis or illness, the coordinator will call the participant to consent for and administer the short-version COVID Questionnaire. If the phone consent is not possible, the coordinator will send the LFU contact letter and the participant will call the coordinator to administer the short-version COVID Questionnaire.

Mail Consent

Prior to the Phase 3 return visit, subjects who were not seen in Phase 2 may be contacted by the Clinical Center via mail to request additional consent and authorization. This additional consent and authorization will request that the subject allow transfer of identifiers from the Clinical Center to the Data Coordinating Center at National Jewish Health. This Mail Authorization provides permission for the Clinical Center to transmit the subject's personal information (social security number, address, secondary contact address) to the DCC. A Personal Contact Information Update form will be sent with the mail consent, asking subjects to update contact information, including secondary contacts and primary care physician. Additionally, the subjects will be invited to sign an "Authorization to Release Protected Health Information" medical record release form.

The participant will consent to the dried blood spot on the phone or by signing a mailed consent form that they will send back to COPDGene Administrative Core with their dried blood spot card. The COPDGene Administrative core will then send the consents to the appropriate site. The mailed consent will have the SID and name of the participant; this information will remain confidential.

This information will be used for central subject tracking, longitudinal follow up, vital status searching, and to obtain cost and utilization information on subjects from Medicare. The mail consent also allows the Clinical Center to seek perimortem medical records and informant interviews about the circumstances around the subject's death.

Phase 3 Options for Subjects Who Have Difficulty with a Visit to Clinical Center

In the event that a previously enrolled subject has moved away from their local Clinical Center and to an area closer to another participating COPDGene Clinical Center, the subject may elect to complete Phase 3 at this closer Center rather than traveling to their original Center. With the subject's verbal approval by phone, their new contact information will be provided from the Clinical Center where the subject had the first visit to the geographically proximate Clinical Center. The subject will sign a new Informed Consent Form and HIPAA with the coordinator at the new study location prior to their Phase 3 visit.

In the event that a subject has financial hardship or has moved a considerable distance from their local Clinical Center, the COPDGene Administrative Core may consider providing financial transportation assistance to this subject for a return visit. The reimbursement for this subject's return visit may include the actual reasonable cost of transportation, lodging, and meal expenses. Subject must provide receipts to Clinical Center for reimbursement. Use of this approach and funding for these expenses will be allocated on a case-by-case basis from the Administrative Core to the Clinical Center.

Subjects who are unable to come to a Clinical Center and are not willing or able to undergo a home visit will be asked if they would be willing to complete study questionnaires over the phone. If the subject agrees, this will be completed. The subject should be compensated $25 for their time and effort in completing the questionnaires over the phone. If the subject is not willing or unable to complete study questionnaires over the phone they will be asked if they would complete a Limited Follow-Up Questionnaire. The Limited Follow-Up Questionnaire will be used to gather important longitudinal information on the subject related to respiratory symptoms, exacerbations, and comorbidities. A research coordinator will conduct this questionnaire with the subject by phone. These subjects may still participate in the bi-annual Longitudinal Follow-Up program by phone or Internet.

Closed Clinical Center

Due to the longitudinal nature of the study, it is important to follow health and vital status on all of the COPDGene subjects. In the event that a clinical center is unable to perform follow-up contacts or visits, the COPDGene Administrative Core will work as an agent for the Clinical Center or provide a contract clinical research organization to manage the follow-up subject visits and contacts per protocol. Some subjects have been consented in Phase 2 to allow for a member of COPDGene Central Administration to contact them for follow-up and this has been tracked by the DCC. The original clinical center must work with the Administrative Core to facilitate efficient transfer of information and study management under the supervision of a local principal investigator and with the approval of the local IRB.

Longitudinal Follow-Up

Subjects will continue to be contacted as they were in Phase 1 and Phase 2, up to four times per year by telephone, mail, or email for up to 10 years after their Phase 3 consent. Questions will be asked about current health status, exacerbations, cancer, new illnesses or medical conditions and current smoking status. If lung cancer is diagnosed, a clinical coordinator may contact the subject via phone or email to ask them to provide medical records to aid in the completion of a Cancer Data Collection Questionnaire, to collect additional data on the lung cancer diagnosis. Subjects may be contacted on no more than three additional occasions per year to inform them of other research studies and to update them about results of the COPDGene study. The longitudinal follow-up contact mechanism is primarily based on automated contacts to subjects via a computer server controlled by the local clinical center in which the clinical center securely uploads subject contact data and social security numbers to a secure server using secure sockets (SSL) technology and 128 bit or greater encryption with an HTTPS protocol. Subjects establish a preference for contact by email and web data entry or automated phone calls with data entry by telephone keypad. Subjects can also request only coordinator-conducted surveys. Subject contact information and identifying information is deleted automatically from the server after the contact is made or at the end of three weeks. The longitudinal follow-up telephone process only includes contacting the subjects. Data collected from all subjects are de-identified and made available to the Data Coordinating Center at National Jewish for storage and analysis. Subjects who fail to respond to automated contacts are contacted by the local clinical coordinator who then asks the questions of the subject and inputs the information obtained into the web-based data collection form. Subjects who become lost to follow-up from the longitudinal follow-up process will be traced by their secondary contacts and searched for in the Social Security Death Master File or National Death Index. Deaths identified from the longitudinal follow-up system are

communicated to the Data Coordinating Center and the local clinical center. The local clinical center initiates collecting further information about the death as described below.

Mortality Assessment

Death is an endpoint of interest that will be analyzed as part of this protocol. The opportunity to assess vital status will occur in one of several ways. It is possible that during the process of longitudinal follow-up, the clinical center will be made aware of a subject's death. However, some subjects will be lost to follow-up with their vital status unknown. For these subjects, staff at the local clinical center or Administrative personnel at National Jewish Health (if subject identifiers have been transferred) will conduct a search to determine vital status using subject's stored social security numbers. This search may include a National Death Index query, Social Security Death Index query and general internet search including obituary postings. Once vital status has been confirmed as dead, the clinical center will obtain additional information regarding the subject's death so that cause of death may be determined. This additional information will include: 1) Informant Interview conducted with next of kin or other close contact of the study participant or the subject's physician, 2) Death certificate, 3) Hospital Discharge Summary if it is determined that the death occurred in hospital or within 3 months of a hospitalization, and 4) Request for recent treating physician records if death did not occur in the hospital.

The above records will be de-identified and transferred to the Data Coordinating Center. A Mortality Adjudication Committee will review these records centrally. The data will be reviewed by the Mortality Adjudication Committee and a final cause of death will be assigned.

VII. BIOSTATISTICAL ANALYSIS

In Phase 1, genome-wide association analysis was performed using genome-wide SNP genotypes obtained in the entire COPDGene study population. In Phase 2, exome chip genotyping, and whole genome sequencing data were obtained. In Phase 3, we will perform both longitudinal epidemiology analysis and analysis of genetic and Omics data.

For longitudinal epidemiological analysis, the primary outcomes for COPD development and progression are changes in FEV_1, CT emphysema [Adjusted Lung Density (PERC15 adjusted for lung volume)], functional small airway disease by PRM, and all-cause mortality. In addition to the "change" variables for lung function and imaging, we will average over repeated measures for each outcome to increase power. Secondary outcome measures include respiratory mortality and respiratory exacerbation frequency, as well as changes in CT airway wall thickness, CT gas trapping, six minute walk distance (6MWD), and health-related quality of life. Longitudinal analyses will be performed with quantitative imaging phenotypes from the Phase 1, 2, and 3 visits. Key predictor variables to be assessed for these outcomes include age, gender, current smoking status (at each visit), pack-years, baseline lung function, baseline CT emphysema and airway measures, respiratory symptoms (e.g., chronic bronchitis, dyspnea), respiratory medications, and co-morbid illnesses (including lung cancer and coronary artery disease). Exacerbation frequency will be assessed as a predictor of primary outcome measures. Transformations of CT and spirometric progression measures from among the Box-Cox family of transformations will be considered to stabilize variance estimates across the ranges of CT measurement values. We will compare the demographic and clinical characteristics of subjects who have follow-up data to those subjects missing due to death and those subjects missing due to

loss to follow-up. Longitudinal analysis will be performed including time-dependent covariates such as change in current smoking status. Time-dependent covariates in these analyses include change in current smoking status and change in pulmonary medications. Disease progression and mortality will be simultaneously assessed to limit potential bias introduced by differential survival. This analytical approach considers the joint distribution of the vector of repeated measures (i.e., longitudinal progression of PFT abnormalities and CT measures) simultaneously with time to event (i.e., mortality) using the method developed by Hogan and Laird(10).

The power to detect differences in 10-year changes between subtypes of COPDGene subjects was assessed for FEV_1, PERC15, adjusted lung density, and % gas trapping. These calculations used the mean and standard deviation (SD) with estimated changes in FEV_1(11), PERC15(12), adjusted lung density, and % gas trapping based on the 5-year follow-up visits. We assumed a significance level of 0.05 and equal variance of the change in each subtype group. These conservative calculations are based on comparing differences in rates of change for COPD-related phenotypes among two hypothetical groups of subjects ranging in size from 250 to 2000, which will be created from the 4500 subjects with follow-up spirometry, clinical measures, and chest CT scans in Phase 3 (**Table 2)**. Using these assumptions, there is adequate power to detect differences between subtype groups with as few as 250 subjects. By 2-sided log rank test, we will have virtually 100% power to detect a 15% difference in all-cause or cause-specific mortality between the slow and fast FEV_1 decline groups in Table 2.

Table 2: Power (%) for Longitudinal Epidemiology Analysis

Outcome	Difference Between Groups over 10 yrs	Sample Size of Each Subtype Group for Pairwise Comparisons			
		250	***500***	***1000***	***2000***
FEV_1, ml	100	97	100	100	100
	200	100	100	100	100
PERC15	4	75	96	100	100
	8	100	100	100	100
Adjusted Lung Density	2	46	75	96	100
	4	96	100	100	100
% Gas trapping	2	70	94	100	100
	3	96	100	100	100

The primary phenotypes for genetic, transcriptomic, and proteomic analysis will be each COPD subtype vs. smoking controls; the quantitative emphysema and airway disease axes; and the change in FEV_1 and adjusted lung density between the 5-year and 10-year visits. In addition, a number of secondary analyses will be performed, including: a) presence/absence of COPD (comparing subjects with GOLD 2-4 COPD to smoking controls); b) cross-sectional measures of CT emphysema, CT gas trapping, CT airway wall area, FEV_1, 6MWD, oxygen saturation, emphysema distribution, and chronic bronchitis; and c) longitudinal measures of exacerbation frequency and change in CT gas trapping, CT airway wall area, and 6MWD. Cross-sectional and longitudinal COPD-related phenotypes will be assessed within each COPD subtype and within the entire study population. Longitudinal imaging and lung function phenotypes will be assessed as both change over time (between the 5 and 10-year visits and across all three visits) and average outcomes over all three visits.

Phase 2 blood RNA-Seq will be used to generate gene, exon, and isoform-level estimates of relative expression abundance. RNA-Seq data will be processed by the CDNM bioinformatics pipeline that addresses sequencing errors and other technical artifacts. Alignment to the human

reference genome will be performed with STAR(13), followed by multiple quantification and analysis options, including DESeq2(14), DEXSeq(15), and voom/limma(16). While isoform-level resolution is most reflective of underlying biology, it is technically challenging to accurately infer isoform levels from short-read fragments(17), and for some applications gene or exon-level quantifications may be preferable to isoform quantification(14, 15). Gene-level quantification of differential expression analysis will be performed using voom/limma, which accounts for the distributional aspects of RNA-Seq data through a weighted regression approach. Identification of differential exon usage will be performed using a novel implementation of DEXSeq adapted for large studies. These tools are all implemented in R/Bioconductor. Isoform quantification and differential expression analysis will be performed using pseudoalignment-based methods such as kallisto(18) and sleuth for isoform quantification and differential expression, respectively. Associations of gene expression to COPD subtypes will be adjusted for covariates, including complete blood count differentials. Surrogate variable analysis will be used to correct for potential batch effects and to remove unwanted sources of variability.(19)

For proteomics analysis, the relationships between the categorical and quantitative outcomes and the predictor variables (~1310 individual plasma protein biomarkers in the SOMAScan panel) are assessed using standard regression models. Covariates in these analyses include age, gender, pack-years of smoking, and current smoking. To control the false discovery rate, the Benjamini-Hochberg method is used to adjust the p-values. These biomarker measurements will be integrated with pQTL genetic variants to improve accuracy of risk prediction.

COPD-related phenotypes will be assessed with whole genome sequencing (WGS) data for possible associations with both rare and common variants. In addition, for each protein and transcript that shows significant differences among COPD subtypes or between cases and control subjects, both *cis* (within 1 Mb) and *trans* (genome-wide) association analysis will be performed using WGS data. Although we will re-evaluate analytical methods for rare variants when our Omics and WGS data become available, our current primary analysis plan is to test single common and rare variants using linear regression or the Firth test, and the Sequence Kernel Association Test (SKAT) method(20, 21) for grouped rare variants. The SKAT framework has been extended in several ways, including with SKAT-O (an optimal combination with a standard burden test) and RC-SKAT which performs well for a combination of rare and common variants. To control for population substructure, we will adjust all analyses using the rare-variant approach based on the Jaccard similarity index for population stratification(22, 23). We will have two primary analyses for *trans*-acting QTLs based on WGS data: 1) Genic regions with a fixed border (i.e., 50kb from both ends of the gene), and 2) sliding windows of variable numbers of SNPs across the genome(24). In addition, we will partition the genome into non-overlapping, adjacent segments to be analyzed with the region-based test. Dr. Lange has developed a computationally rapid method for partitioning the genome based on the haplotype structure(25).

To determine power to detect genetic associations, we used simulation studies (in C) to estimate the statistical power of SKAT for grouped rare variants with variable directions of effect on COPD subtypes. We assumed COPD subtypes would include 25% of the affected population, with 20% of all causal variants having opposite/protective effects. We assumed overall odds ratios for the region of 2.25 and 2.5 for all variants combined. We used a 10kb region size, based on a simulated haplotype spanning 200kb, and a genome-wide significance level (assuming a Bonferroni genome-wide correction for 10kb non-overlapping windows) of $2x10^{-8}$. The number of tested loci will be reduced by phasing the entire genome and removing redundant variants. We

will further increase statistical power by performing permutation testing at the genome-level. We examined only alleles with minor allele frequency <5%. Power estimates shown in **Table 3** for non-Hispanic White (NHW) subjects are based on 1000 replicates. Statistical power for the NHW group analysis in Aim 2 will be acceptable for a rare variant analysis of genomic regions where a majority of the SNPs are true disease susceptibility loci. Power for African American (AA) subjects is above 80% for an odds ratio of 2.5.

Table 3: Power to Detect Rare Variant Associations to COPD Subtypes within the COPDGene NHW Population

	% of Variants in Region That Are Disease Susceptibility Loci			
Genetic Effect Size	**20**	**40**	**60**	**80**
Odds ratio of 2.25	24.3	43.1	68.2	82.7
Odds ratio of 2.5	32.1	58.8	84.9	95.4

We will also perform epidemiological and Omics analysis in 800 subjects from the Short-Term Follow-Up Study Population. The relationships between the outcomes of interest (FEV_1 and adjusted lung density) of each of the three high risk groups compared to the reference group will be assessed using linear mixed models. Covariates in these analyses will include risk group, visit, age, gender, pack-years of smoking, current smoking status, and baseline FEV_1 % predicted. The relationship between changes in FEV_1 and changes in adjusted lung density will be assessed by including time-varying as well as subject mean values in the model as predictors. An unstructured covariance structure (or other appropriate structure) will be used in the model to account for repeated measures. We will adjust for multiple statistical testing by requiring $p < 0.01$ to demonstrate any significant association.

The power estimates to detect differences in change in FEV_1 or adjusted lung density for the Short-Term Follow-Up Study are shown in **Table 4**. Two rapid progression groups will be tested for change in FEV_1 (High COPD Progression Score and Paraseptal Emphysema) and two rapid progression groups will be tested for change in adjusted lung density (High Risk Airway Disease Axis and Paraseptal Emphysema). Based on the five-year changes in those groups compared to non-high risk subjects, 200 subjects per group will provide excellent power to detect differences between the rapid progression and non-high risk groups over one year. Power to detect differences for RNA-Seq and proteomic biomarkers between 200 rapid progression and 200 non-rapid progression subjects was assessed. We will have 80% power to detect effect sizes > 0.6 standard deviations, which will be adequate to detect useful biomarkers.

Table 4: Power (%) for Short-Term Follow-Up Study

Outcome	Rapid Progression Group	Sample Size of Each Short-Term Follow-Up Group		
		100	***200***	***300***
FEV_1, ml/yr	High COPD Progression Score	100	100	100
	Paraseptal Emphysema	72	95	99
Adjusted Lung Density, per yr	High Risk Airway Disease Axis	100	100	100
	Paraseptal Emphysema	100	100	100

VIII. RISKS AND DISCOMFORTS

The only drug administered in this study is albuterol, as part of the pulmonary function testing. Two puffs of albuterol (180 mcg) are given via metered dose inhaler, with a spacer. After waiting 20 minutes, spirometry is performed. This is a one-time dose only. Albuterol is a commercially available, FDA-approved drug. The common side effects associated with albuterol are transient tachycardia, tremulousness, and nervousness.

Subjects may become short of breath during the six-minute walk test as it is designed to assess subjects' maximal exercise capacity. There is a potential risk of syncope, light-headedness and fainting during these tests; these are uncommon. There is a potential for the subject to develop chest pain or cardiac arrhythmias; these are uncommon. There is a remote risk of heart attack or death during this test. All subjects in this study have previously had a walk test performed successfully without adverse events as part of the COPDGene enrollment visit, and walk tests were also performed during the 5-year visit.

Subjects may become short of breath or have generalized muscle fatigue during or after the sit to stand test, as it is designed to assess subjects' muscle strength. There is a potential risk of syncope, light-headedness and fainting during these tests; these are uncommon. There is a potential for the subject to develop chest pain or cardiac arrhythmias; these are uncommon. There is a remote risk of heart attack or death during this test.

From spirometry and DLco, subjects may become short of breath. Syncope, light-headedness, and fainting may develop during this test; these are uncommon. Occasionally during or after pulmonary function testing, subjects may become temporarily more short of breath. Subjects who are using oxygen may become short of breath when the oxygen is removed. However, this should rarely occur since oxygen will only be discontinued for 10 minutes and the oxygen saturation will be continuously monitored during this time.

There is a possible risk in questionnaire administration from inadvertent disclosure of medical history information. There is also a risk of loss of confidentiality. These potential risks are guarded against by maintaining completed questionnaires in a locked filing system in a locked room at the Clinical Centers, password-protected computers, and using secure transmission of information to the Data Coordinating Center (DCC). Pulmonary function and questionnaire data,

identified by study ID number only, will be transferred by secure internet connection (with 132-bit encryption) to the Data Coordinating Center at National Jewish Health in Denver. Identifying information will be sent from the Clinical Centers to the DCC, but this identifying information will be stored separately from clinical and genetic information collected in COPDGene. Study personnel at Clinical Centers and the DCC will be required to meet local requirements for training in protection of confidentiality.

Potential risks of blood drawing are hematoma at the skin site and minimal pain of venous puncture.

Risks associated with the dried blood spot kit include slight discomfort when using the lancet (people have described the feeling as a slight sting – much less than you would experience in a typical blood draw) and, as with any cut, there is a small risk of infection and/or bruising. However, the process and products in the kit are used routinely in a wide range of healthcare applications, including measurement of blood glucose in diabetes. The equipment is sterile and following the instructions provided in the kit (like cleaning the area) will minimize infection risk.

Radiation exposure in the chest CT scan could theoretically increase the risk of cancer. Exposure of pregnant women to CT scan radiation could be harmful to the developing fetus. To minimize the likelihood of exposing pregnant women to CT scans, females of child-bearing potential will be asked if they are pregnant or have the possibility of being pregnant before the chest CT scan. CT scans will not be performed for women who state they are pregnant or that they may be pregnant; other pre-menopausal women will undergo urine pregnancy testing before a chest CT scan is performed. Chest CT scans could identify pulmonary nodules that may require follow-up outside of this study. Such pulmonary nodules could be curable lung cancers (a benefit) or scars/prior granulomas that could require additional radiation exposure or even surgery (a risk).

The maximum amount of radiation exposure, during the chest CT scan is approximately 7 mSv at each study visit. The radiation dose differs with body size; thinner subjects will have less than this amount of radiation. The average amount of background doses of radiation that the general population is exposed to in the United States is 3 mSv per year. Thus, the maximum amount of radiation subjects will receive from each study visit is equivalent to about two and one third years of normal background radiation.

There is a risk that the depression questionnaire (Hospital Anxiety and Depression Scale, HADS) will identify subjects with potential clinical depression. The HADS cannot diagnose clinical depression; the diagnosis of clinical depression can only be made by a health care professional. To assure these subjects have further evaluation and therapy if appropriate, subjects will be told if their score (16 or greater) (14) indicates possible depression and will be informed to seek further care from their personal physician. The HADS score will be immediately available to the Research Coordinator and if abnormal the Research Coordinator will inform the subject in person before the study visit is ended. In addition, this information will be mailed to the subject's primary care provider who will be asked to perform a clinical evaluation to determine the possible need for further evaluation or treatment for depression. The DCC will collect information from the study coordinators to verify that subjects and their physicians have been informed about such scores.

There are no costs associated with participating in this study. Participants will be compensated for their time and expenses in this study as follows. Participants will be compensated $125,

pending local IRB approval, for completing the Phase 3 study visit. The subjects who cannot have a visit may be offered compensation of $25, for time and effort required of completing all questionnaires over the phone.

This study is designed to be a national resource for scientific investigations. As such, medical information, genetic information and biosamples will be provided to monitored data repositories such as dbGaP as one example and other Biorepositories in order to make the study data available to other investigators, with appropriate safeguards. Other researchers interested in using such information for scientific investigations will be required to apply to the COPDGene Executive Committee for permission to access the data for studies that have received local IRB approval and with requirements to maintain subject confidentiality. Subject-identifying information will not be transmitted to other investigators. A Certificate of Confidentiality has been obtained for the COPDGene study to provide additional protection for study participants.

IX. POTENTIAL BENEFITS

There are no expected benefits to the study participants. Improved understanding of COPD has occurred; COPDGene has generated over 230 scientific publications. As noted above, CT scans could identify pulmonary nodules, early lung cancer or other abnormalities that may require follow-up outside of this study.

X. MONITORING AND QUALITY ASSURANCE

This is an observational longitudinal investigation without a therapeutic intervention. It is expected that there will be deaths in both control and COPD subjects enrolled in this study that are not related to study procedures. It is expected that there will be hospitalizations from a variety of causes not related to study procedures including but not limited to newly discovered disorders, acute disorders requiring surgery, pre-existing conditions, and exacerbations of underlying COPD. Subjects may expire due to pre-existing or new diseases including cancer, cardiovascular conditions, and COPD. These are anticipated events that are not related to this investigation. These events will not be prospectively collected as part of the current study and thus will not be reported to IRBs. Adverse events for the purposes of this study will only be those that are directly related to study procedures done during the study visit(s). There are no expected Serious Adverse Events in this study related to study procedures. At each Clinical Center, subjects will be observed for the development of tremulousness, and nervousness following bronchodilator medication. Unexpected Adverse Events related to study procedures will be reported to the IRB of the Clinical Center and to the Executive Committee. An Observational Safety and Monitoring Board (OSMB) has been appointed by the National Heart, Lung, and Blood Institute and will continue to oversee this study. All protocol deviations and adverse events will be reviewed by the OSMB, and the OSMB will vote on modifying the study if needed.

Quality assurance of spirometry data will be insured by the Pulmonary Function Core at National Jewish Health and in Utah, which will review de-identified spirometry data from each study participant. Quality assurance of CT scans will be analyzed by the Imaging Core in Denver. Questionnaires and other data will be quality controlled by the Data Coordinating Center in Denver.

As noted above we anticipate that some subjects may expire during the next phase of this study due to a combination of pre-existing disease and the onset of new conditions. These events are not anticipated to be related to the study visit; however, they provide important information about the natural history of COPD and other smoking-related conditions. We will monitor and collect information about deaths in the cohort but will not report them to IRBs as study-related events unless they occur during a study visit or within twenty-four hours of the study visit and are judged to be related to a study procedure.

The COPDGene Administrative Core will hire National Research Coordinators. They will visit each Clinical Center periodically to review site facilities, monitor study procedures and record keeping, assist with continuing education of local coordinators to continue our excellent track record of high quality study data and completeness, and as needed and when locally approved, assist in conducting study visits. Clinical centers will be responsible for providing study records and logs for review to the National Research Coordinators.

XI. DATA STORAGE AND DISTRIBUTION

The COPDGene study has nine study cores and twenty participating clinical centers. Clinical centers send data to the Data Coordinating Center (DCC) at National Jewish Health, the Biorepository at Brigham and Women's Hospital, the Pulmonary Function Testing Center (PFT) at NJH and to the Imaging Center at NJH.

The Administrative Center oversees and coordinates all study activities. The DCC maintains confidentiality of all protected health information collected from study participants and stores subject data. The Administrative Center releases data and Biospecimens to internal and external study investigators. Internal Investigators consist of co-investigators at the twenty clinical centers and at the study centers. The Ancillary Studies and Publications Committee oversee distribution of data and Biospecimens to external investigators, outside of the COPDGene study.

The Imaging Center receives subject CT scan image files. Data and image files including study dates are shared between three different sites, National Jewish Health, University of Iowa and Brigham and Women's Hospital, under the Imaging Center.

The Pulmonary Function Testing (PFT) Center receives subject data from twenty clinical centers via a file depot located at National Jewish Health provided by the DCC. The PFT Center is located in two locations, at National Jewish Health and reviewed by Dr. Robert Jensen as a contract consultant, at the University of Utah.

The Biorepositories, which collect, store, and distribute blood samples from the twenty centers, are located at Johns Hopkins University and Brigham and Women's Hospital. The Phase 3 samples will be stored at the biorepository at Brigham and Women's Hospital. All samples are stored with a code but no other identifiers. Dates of blood collection are recorded in the DCC and are received but not stored at the Biorepository. Blood samples from the Biorepository may be shared with internal study investigators as well as with external investigators, as approved by the Ancillary Studies Committee.
If collected in the future, FFPE tissue blocks will also be stored in an established COPDGene Biorepository at Brigham and Women's Hospital, or at National Jewish Health.

The Genetic Analysis and the Sequencing and Bioinformatics Centers are located at Brigham and Women's Hospital, University of Colorado, Denver and Johns Hopkins University. These centers receive DNA samples and genetic data for analysis. Samples and data are coded and have no other identifiers associated with them.

The Biomarkers Center is located at National Jewish Health and works collaboratively with the Biorepository sharing biospecimens to be used for analysis of biomarkers. Data will include dates of sample collection.

The Subtyping Center is made up of investigators from the twenty clinical centers, who meet by teleconference and collaborate on data analysis. Data shared to this Center will include geocodes and location to the level of zip codes.

The Mortality Adjudication Center is located at National Jewish Health and is closely linked to the Administrative Center. They will receive death certificates, subject medical records and when the subject has consented to have personal identifiers transmitted to the DCC staff for the Mortality Adjudication Center will contact next of kin for informant interviews and request death certificates.

The Epidemiology Center is located at the University of Colorado, Denver. The Epidemiology Center participates in data analysis of phenotypes, genetic data, and imaging data. These data may include subject identifiers, including dates and geocodes (represents a geographic location).

The Principal Investigators for the study, the head of the DCC, the director of the Quantitative Imaging Lab, the Director of the Sequencing Center and Biorepository, Head of the Epidemiology Center, Head of Genetic Analysis Center, the Clinical Centers Director and the Associate Director for COPDGene, in consultation with the COPDGene Executive Committee, manage the central functions of the study. This team and the COPDGene® Investigators seek to encourage appropriate collaborative relationships with outside investigators to advance scientific knowledge and maximize the value of the study.

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The Phase 1 and Phase 2 COPDGene protocol items are included as appendices.

Appendix A: Phase 1- Completed

I. SUBJECT SELECTION: Phase I (Completed)

We are recruiting 12,000 smoking and non-smoking subjects.

Subject Inclusion/Exclusion Criteria
A total of 12,000 non-Hispanic White and African-American subjects will be recruited. These subjects will be classified into four groups:
(1) Up to 4500 subjects with COPD GOLD Stages 2 through 4,
(2) Up to 4500 control subjects – current or former smokers without airflow limitation,
(3) Up to 1500 subjects with minimal airflow limitation (COPD GOLD Stage 1) and including smokers unclassified by GOLD criteria (26, 27),
(4) Up to 1500 non-smoking control subjects, 500 African-American subjects and 1000 non-Hispanic White subjects, with no smoking history and no airflow limitation.

Subjects will be collected at 21 clinical centers in the United States.

General Inclusion and Exclusion Criteria for All Study Subjects
The following criteria will be required on ALL smoking and non-smoking study subjects in the first study visit:

Inclusion Criteria
- Age 45-80 years (Age 45-85 for non-smoking subjects)
- Non-Hispanic Whites and African Americans

Exclusion Criteria
- Other concomitant respiratory disorder (such as, but not limited to, diffuse bronchiectasis, cystic fibrosis, or interstitial lung disease)
- Lung surgery with removal of a lobe or more (including lung volume reduction and lung transplantation)
- Lung cancer, known or suspected
- Bronchoscopic lung volume reduction
- Pregnancy or suspected pregnancy
- Uncontrolled cancer, as defined as ongoing radiation therapy, ongoing chemotherapy, narcotics for pain control, or known metastatic disease
- History of radiation therapy to the chest (other than radiation for breast cancer)
- Use of antibiotics and/or systemic steroids (new prescription or increased dose) for a COPD exacerbation or any lung infection within the last month
- Inability to use albuterol
- First or second degree relative (parent, brother, sister, daughter, son, aunt, uncle, nephew, niece, half-sibling, grandparent, grandchild) of a subject enrolled in COPDGene®
- Subjects who indicate they are in more than one racial category
- Metal objects that may interfere with chest CT quantification including presence of a cardiac pacemaker, defibrillator, metal prosthetic heart valve, metal projectile or metal weapon fragment (bullet, shrapnel, shotgun shot) or metal shoulder prosthesis

Subjects unable to perform spirometry due to:

- chest or abdominal surgery in the past three months
- a heart attack in the last three months
- detached retina or eye surgery in the past three months
- hospitalization for any other heart problem in the past month

Participation in the ECLIPSE study
Inability to provide telephone contact number(s) and two additional contacts
No place of permanent residence of three months or more

Each of the four subjects groups contains specific criteria that define the distinct group. The criteria are relevant to the clinical and epidemiological categorization of the four study groups.

COPD Subjects:

Additional Inclusion Criteria

Smoking history of ≥ 10 pack-years
Diagnosis of COPD (post-bronchodilator $FEV_1/FVC < 0.70$) Stages 1, 2, 3 and 4 by GOLD criteria (26)

Additional Exclusion Criteria

Smoking history of < 10 pack years

The diagnosis of COPD includes airflow limitation on spirometry and history of risk factors (most commonly cigarette smoking) known to cause COPD (26). We will use NHANES predicted spirometry values obtained in the United States (28). There are no uniform criteria for the amount of cigarette smoking required for the diagnosis of COPD. We have chosen a threshold for cigarette consumption (10 pack-years) to ensure that all subjects have a substantial environmental stress to differentiate those who have an abnormal pulmonary response to cigarette smoke and those who do not have such a response. Review of medical records of patients with COPD frequently demonstrates a physician-listed diagnosis of asthma. These subjects will be included in the COPD groups if they have evidence of airflow limitation that is not fully reversible using the GOLD (Global Initiative on Obstructive Lung Disease) criteria (26). The interactions of asthma with COPD are common and complex. Elimination of asthma patients with fixed airflow limitation would inappropriately bias the findings of this study.

We will employ the FVC as the primary measure of lung volume since this allows comparisons to previous large-scale epidemiologic investigations. Normal values have been published and this maneuver is widely used. However, we will also collect the FEV_6, a more recently advocated index that may be easier for some patients to accomplish and reduce adverse effects of spirometry.

Subjects with known or suspected lung cancer will be excluded; if there are a substantial number of subjects with lung cancer in this study, then we may detect genes associated with lung cancer rather than with COPD. Subjects with a prior history of lung cancer, even if successfully resected and cured will be excluded. Subjects who may in the near future have a resection of a lesion suspected to be lung cancer will be excluded, but they may be included at a later time if the lesion proves not to be cancer and the resection removes less than one lobe of the lung.

Subjects with uncontrolled cancer of any type will be excluded because the cancer or cancer treatment may alter lung function and thus misclassify the respiratory status of the subject, and this cohort is being designed for long-term follow-up.

We will exclude patients with recent use of antibiotics and/or systemic steroids for a COPD exacerbation since such subjects may have an ongoing inflammatory pulmonary process related to an infection that will impair our ability to characterize and phenotype patients in their usual stable state. These patients can be re-screened 30 days after cessation of antibiotic or corticosteroid use.

GOLD Unclassified Subjects:

Additional Inclusion Criteria

Smoking history of ≥ 10 pack-years

Spirometry (Post-bronchodilator $FEV_1/FVC \geq 0.70$, $FEV_1 < 80\%$ predicted)

Additional Exclusion Criteria

Smoking history of < 10 pack years

Subjects meeting the required smoking history parameter but do not fall into either COPD or smoking control categories will be included as GOLD Unclassified subjects. These subjects will be grouped based on the occurrence of a normal FEV_1/FVC accompanied by a presence of reduced air flow (post-bronchodilator $FEV_1/FVC \geq 0.70$, $FEV_1 < 80\%$). To date, we have found approximately 10-12% of smoking subjects fall into this category. The inclusion of the Unclassified smoking group will provide additional and possibly novel information regarding the categorization, clinical presentation, and progression of COPD in smoking populations. Since this group is already present within the current subject pool, these subjects will be clarified as a distinct subject group.

Smokers without COPD

Additional Inclusion Criteria

History of cigarette smoking ≥ 10 pack-years

Post-bronchodilator $FEV_1/FVC \geq 0.70$ and $FEV_1 \geq 80\%$ predicted.(28)

Additional Exclusion Criteria

Smoking history of < 10 pack years

Some potential subjects may have exclusionary criteria that only temporarily limit their enrollment in COPDGene®, such as recent use of antibiotics or corticosteroids. Such subjects with temporary exclusion may be re-screened and enrolled at a later time when these features are no longer exclusionary.

Subjects who have been given a diagnosis of COPD by a health care professional, but who have normal spirometry, will be enrolled as smoking control subjects. The diagnosis of COPD may have been incorrectly given to a patient without confirmation by spirometry. For this study, the diagnosis of COPD or lack thereof (based on the objective presence or absence of airflow limitation) will be determined by the post-bronchodilator spirometry performed as part of this study.

Sources of subjects will vary from center to center, but will likely include inpatients and out-patients at the centers, spouses and friends of subjects with COPD, patients in primary care practices, local patient support and educational groups, and local and national COPD voluntary organizations (such as the COPD Foundation and American Lung Association).

Subjects should not primarily be recruited from sources that include a high prevalence of asthmatics such as asthma clinics or asthma patient groups. While subjects with asthma are not

excluded in either control or COPD subjects in order to assure similar inclusion/exclusion criteria in both populations, this study is not designed as a study of the genetics of asthma. Thus subjects who have asthma as their primary respiratory disease should not be targeted for recruitment.

Although the primary focus of this project is COPD and COPD-related phenotypes, subjects will also be informed that this cohort may be used to study the genetic and environmental determinants of other smoking-related illnesses such as lung cancer and coronary artery disease and, with their permission on the consent form, other disorders that are not smoking-related.

Non-smoking Controls

Additional Inclusion Criteria

No smoking history as defined by less than 100 cigarettes smoked in a lifetime
No airflow limitation (Post-bronchodilator $FEV_1/FVC \geq 0.70$, $FEV_1 \geq 80\%$ predicted)

Additional Exclusion Criteria

Smoking history of more than 100 cigarettes smoked in a lifetime
Smoking history of more than 52 cigars smoked in a lifetime
Smoking history of more than 12 oz. of pipe tobacco smoked in a lifetime
Any history of physician-diagnosed respiratory disease

One hundred and eight subjects with no smoking history and no airflow limitation were included as a reference population for comparison with those affected by smoking exposure. These subjects will offer information on the processes involved in normal lung aging. Non-smoking control subjects are critical for supporting genetic and pathological findings within smoking and diseased subject groups by acting as a baseline for normal pulmonary physiology within a genetically mixed population.

Subject Phase 1 Actual Enrollment:

Phase 1, which was performed from November 2007 until July 2012, enrolled 10,371 subjects. Of these subjects 3438 were African American and 6933 were Non-Hispanic White. Enrolled subjects classified by severity of lung disease were as follows:

Smoker Controls – 4391
GOLD 2 - 4 Subjects – 3692
GOLD Unclassified – 1258
GOLD 1 Subjects – 795
Non-smokers controls – 108
Lack final GOLD classification due to failed spirometry – 63
Significant ILD or bronchiectasis noted on CT scan, excluded from analysis – 64

All of the enrolled subjects had at least a 10 pack-year smoking history (except the non-smoker group) and were between 45 and 80 years of age. Enrollment of up to a total of 1500 non-smoker controls is still anticipated.

II. SUBJECT ENROLLMENT

Phase 1
This project will recruit a total of 12,000 subjects over five years from twenty-one clinical study centers. We anticipate recruitment will be completed early in the fifth year of the proposed study, allowing time for the genetic and epidemiologic analyses to occur by the end of the fifth year. Each Clinical Center will be expected to, on average, recruit approximately 160 subjects in each of the first four years meeting the program's defined recruitment goals for recruitment among both racial/ethnic classes, an approximately equal division of men and women, and with COPD subjects equally distributed among GOLD grades 1, 2, 3 and 4. The Executive Committee with the help of the Steering Committee will set and monitor goals for subject recruitment from each Clinical Center. The Administrative Core will have the right to modify recruitment goals among the centers to meet the overall goals of the project with respect to numbers of subjects recruited, minority subjects, or the balance of subjects in GOLD stages. The Administrative Core may incorporate additional qualified Clinical Centers if needed to meet recruitment goals.

Potentially eligible subjects who contact study staff or are referred by a health care professional may undergo screening either in person or by telephone to determine if they are likely to meet appropriate inclusion and exclusion criteria and to schedule the study visit to the Clinical Center. The study protocol will be discussed in detail during this screening encounter. A log will be maintained at each Clinical Center indicating the number of subjects who fail this initial screening. This screening log will be maintained at the Clinical Center and will not be transmitted to the Data Coordinating Center to assure confidentiality and protection of human subjects.

Before the study visit begins, written informed consent will be obtained by one of the study staff members. A physician investigator from the Clinical Center will be available to answer any questions regarding the informed consent process. Investigators may obtain consent from their own patients.

III. PHASE I STUDY PROCEDURES (Completed)

There will be one to two study visits for the majority of subjects depending on whether a separate visit is needed to schedule a chest CT scan. In some cases, subjects may have a third visit to re-collect information that does not meet quality control criteria. For example, if spirometry does not meet quality control criteria as judged by the Pulmonary Function Core, the subject may be called to schedule an additional visit to repeat the test.

During their research study visit, the following procedures will be performed:

1. Informed consent will be obtained prior to any other study procedure.
2. Prior to any other evaluations, an Eligibility Questionnaire will be administered to determine if potential subjects meet inclusion/exclusion criteria. This questionnaire is located on the protected study web site and will provide a check on whether subjects meet inclusion and exclusion criteria.
3. Contact Information will be collected from the subject. Name, home address, phone number, cell phone, email address, date of birth and social security number will be collected from the subject. The purpose of this information is to maintain contact with the

subjects up to four times a year for the purposes of longitudinal follow-up. Similar contact information (but not social security number) will be collected for two other individuals likely to know the subject's whereabouts, at least one of whom is a relative not living with the subject. The purpose of this information is to locate subjects who have moved to a different home and have a different address and/or phone number and ascertain vital status of the subject.

4. Safety Assessment. A Safety Assessment questionnaire will be administered to subjects. They will be asked if they have a history of adverse events with the use of albuterol prior to administration of this inhaled bronchodilator. This safety assessment will also be used to ascertain potential cardiac disorders prior to albuterol administration and the walk test. If the questionnaire uncovers known or potential adverse effects of albuterol or potential cardiac disorders, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a six-minute walk test.
5. Physical Assessment will be performed on all subjects: A limited assessment will be performed including height, weight, blood pressure, heart rate, and room air oxygen saturation by pulse oximetry with the subject seated at rest. In subjects using supplemental oxygen, the oxygen will be withheld and subjects will breathe room air for 10 minutes prior to recording oxygen saturation. If resting saturation falls below 82% or the subject becomes dyspneic prior to 10 minutes without oxygen, oxygen therapy will be restarted as a safety precaution. If heart rate is less than 50 or greater than 110 or if the blood pressure is greater than 170/100, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a walk test.
6. Pre and post-bronchodilator spirometry will be performed on all subjects. The maneuvers are performed in a standardized manner. To obtain three acceptable measures, the technician may ask the subject to perform up to eight attempts. Spirometry is performed before and then repeated twenty to thirty minutes following the administration of two puffs (180 mcg) of albuterol from a metered dose inhaler with a spacer. Spirometry will be performed with the subject in the seated position with a nose clip in place. Inspiratory capacity will also be measured post-bronchodilator.
7. Standardized questionnaires will be completed by all subjects that meet entry criteria to assess respiratory history and symptoms, smoking history, family history, and other medical history. These questionnaires include a modified version of the American Thoracic Society/Division of Lung Diseases Respiratory Epidemiology Questionnaire (29) to assess respiratory symptoms and the St. George's Respiratory Questionnaire to assess health-related quality of life. Medications and oxygen use will also be recorded. Questionnaires can be administered by the method judged to be most convenient at each Clinical Center. Questionnaires may be interviewer-administered or self-completed on either a paper copy or directly on a computer pdf file. Completion of all questionnaires is expected to take between 45 and 90 minutes.
8. Blood is drawn from all subjects for DNA (genetic association studies) and serum and plasma (for measurement of other proteins potentially related to COPD and other diseases). A total volume of approximately 40 ml of blood will be drawn for this study. Blood samples will be stored at the Johns Hopkins COPDGene® Biological Repository.
9. Six-Minute Walk Test will be performed on all subjects to determine exercise capacity (30). This will be used to calculate the BODE score. Subjects will be asked questions to

assure they do not have significant or occult heart disease prior to the test to assure subject safety.

10. Chest CT Scan will be performed to assess for emphysema and airway disease in all subjects. An inspiratory chest CT scan will be performed with a radiation dose of 200 mAs to provide thorough assessment of small airway wall thickness and emphysema. An expiratory chest CT scan will be performed of lower dose (50 mA) to assess for air trapping. If a clinical chest CT scan with an appropriate CT protocol and data storage has been performed within 6 months, that clinical CT scan may be used for this study with prior approval of the Imaging Committee that the scan algorithm used meets study criteria and can be analyzed appropriately. If a CT scan may not be scheduled on the same day as the study visit, the scan must be performed within 6 months after the visit. All COPD, Unclassified, and control subjects will be required to have a chest CT scan. If the chest CT scan is done on the same day as the post-bronchodilator spirometry, the scan should be scheduled as soon as possible after albuterol. The time and date of the last bronchodilator prior to the CT scan will be captured.

11. A urine pregnancy test will be performed on all premenopausal women prior to the chest CT scan.

12. Medical Record Review: In subjects with COPD , medical records will be reviewed when available with subject permission to obtain information within the last year of pulmonary function tests (including lung volumes and diffusing capacity) and oxygenation (arterial blood gases).

13. Longitudinal Follow-Up: Subjects will be contacted up to four times a year by telephone, mail, email, and/or newsletter to assess survival status and respiratory illnesses for up to 10 years.

14. Linking to other studies: Subjects with COPD will be asked if they are currently participating or have participated previously in other COPD NIH and non-NIH clinical trials such as the NHLBI COPD Clinical Research Network MACRO or LEUKO studies, NHLBI Long-Term Oxygen Therapy Trial (LOTT), National Lung Cancer Screening Trial (NLST), GlaxoSmithKline International COPD Genetics Network, Boston Early-Onset COPD Study, NHLBI Lung Tissue Research Consortium (LTRC), NHLBI Lung Health Study, and NHLBI SPIROMICS Study. Subjects will be asked if their clinical and genetic data can be linked to the results of these other studies and future investigations in order to investigate the genetic associations between genotype data from this study with their outcomes in other trials.

After the genotyping of candidate genes, including alpha 1-antitrypsin (AAT), is performed, subjects who elected to learn about abnormal AAT test results, when IRB approved, will receive them. These results may not be available for several years after the blood samples are obtained. For newly diagnosed PI ZZ subjects, the Principal Investigator of the Clinical Center will telephone those subjects first, then send a follow-up letter. Subjects with other abnormal PI types will be informed by mail. Participants will be informed that the AAT test results are based on research laboratory test results and should be repeated in a clinical laboratory. If subjects provide permission, these results will also be communicated to their physician.

The questionnaire and pulmonary function test results will be stored in a locked filing cabinet at each Clinical Center. Questionnaire and pulmonary function data, identified by study ID number

only, will be transmitted to the Data Coordinating Center in Denver by secure internet connection with 132-bit encryption. Blood samples and CT scans will be transmitted to the appropriate Biorepository and Imaging Cores by overnight delivery service.

Appendix B- Phase 2 (Year 5 and Year 1 for New Nonsmokers)- Completed

I. COPDGene Phase 2 Return Visit

In addition to the cross-sectional subject data collected in the baseline COPDGene study visit, disease progression and incidence of COPD in smokers are important additional endpoints for genetic association studies. We have completed enrollment of 10,300 subjects as the first phase of this project and now propose to invite these subjects to return for a second evaluation five years after their initial visit to assess disease incidence and/or progression in COPDGene subjects.

The primary goals of COPDGene Phase 2 are: 1) to identify new genetic loci that influence the development and/or progression of chronic obstructive pulmonary disease (COPD) and COPD-related phenotypes, and 2) to reclassify COPD into subtypes that can ultimately be used to develop effective subtype-specific therapies and prevention. The reclassification of COPD will be done using imaging, clinical and physiologic characteristics, longitudinal progression, long-term outcomes, and genetics.

In addition to identifying COPD genetic determinants, this program will characterize the natural history of COPD and identify well-characterized COPD subtypes. Improved understanding of COPD subtypes and genes controlling susceptibility to COPD could lead to novel patho-physiological insights, refined diagnostic criteria, and new treatment approaches. Moreover, the availability of comprehensive genetic data and longitudinal data on a large biracial group of smokers will be an invaluable national resource for other investigators.

New Non-Smoker Controls

To reach our Phase 1 goal of up to 12,000 subjects, we plan to enroll up to an additional 1,500 subjects in Phase 2. We will target enrollment of additional non-smokers without lung disease in Phase 2 of COPDGene. By providing insight into the normal effects of aging in healthy subjects, these subjects will allow us to correctly interpret the chest CT scans, lung function, health status and other features of our current cohort. For example, aging is associated with loss of lung elasticity, a characteristic feature of emphysema. Without knowledge of the effects of age, gender and race on the lungs of healthy subjects, we cannot accurately determine whether CT scans of subjects with a smoking history are normal or abnormal, or assess disease severity.

II. SUBJECT SELECTION – Phase 2

Return Visit

This project will invite all 10,371 subjects who successfully completed Visit 1 using the same 21 clinical study centers for a second study visit. Visit 2 will be scheduled five years (+ / - 3 months) after Visit 1. However, late visits are permitted if the subject becomes available for a Phase 2 visit more than three months after their five-year anniversary.

A key component of the project is to establish contact with all of the previously enrolled subjects or confirm the subjects are deceased. Clinical centers will utilize stored contact information including secondary contacts to contact previously enrolled subjects.

Eligible subjects will be contacted by mail, email and/or phone and invited to participate in Visit 2 at the Clinical Centers. The study protocol will be discussed in detail during this screening encounter. Before the study visit begins, written informed consent will be obtained by one of the study staff members. A physician investigator from the Clinical Center will be available to answer any questions regarding the informed consent process.

New Non-Smoker Control Subjects

Additional subjects without a smoking history will be enrolled in Phase 2 to meet the target of up to 1,500 subjects noted above. Subjects will be recruited from family members and friends of COPDGene enrolled subjects; this primary targeted recruitment will help assure age, race and gender distribution of control subjects is similar to the enrolled COPDGene subjects. If necessary, control subjects will also be enrolled from the community and physician offices using informational brochures and advertisements.

Subject Inclusion/Exclusion Criteria for Non-smoker Controls

Inclusion Criteria

- Age 45-85 years (for non-smokers)
- Non-Hispanic Whites and African Americans
- No smoking history as defined by less than 100 cigarettes smoked in a lifetime, less than 52 cigars smoked in a lifetime, and less than 12 oz. of pipe tobacco smoked in a lifetime

Exclusion Criteria

- Respiratory disorders (including, but not limited to, COPD, asthma, bronchiectasis, cystic fibrosis, or interstitial lung disease)
- Any lung surgery
- Lung cancer
- History of Pneumothorax
- Pleural procedure (including, but not limited to, chest tube placement, pleural surgery)
- History of any pulmonary procedures: diagnostic bronchoscopy, lung biopsy, except research bronchoscopy
- Uncontrolled cancer, as defined as ongoing radiation therapy, ongoing chemotherapy, narcotics for pain control, or known metastatic disease
- History of radiation therapy to the chest (other than radiation for breast cancer)
- Subjects receiving treatment for active TB
- Severe cirrhosis
- Renal disease requiring dialysis
- Pulmonary Hypertension
- Congestive heart failure
- Inability to use albuterol
- First or second- degree relative (parent, brother, sister, daughter, son, aunt, uncle, nephew, niece, half-sibling, grandparent, grandchild) of a subject enrolled in COPDGene
- Subjects who indicate they are in more than one racial category
- Subjects who do not authorize the storage of personal identifiers (social security number, name, address, phone number) in the COPDGene Data Coordinating Center
- Metal objects that may interfere with chest CT quantification including presence of a cardiac pacemaker, defibrillator, metal prosthetic heart valve, metal projectile or metal weapon fragment (bullet, shrapnel, shotgun shot) or metal shoulder prosthesis
- Participation in the following studies: COPDGene Phase 1, ECLIPSE, Boston Early-Onset COPD Study, Boston COPD Exacerbations Study, Denver Genetics Study,

GlaxoSmithKline International COPD Genetics Network, NCI: National Lung Screening Trial, NIH: COPD Clinical Research Network: Macrolide and Leukotriene Trials, NIH: Long-Term Oxygen Therapy Trial, NIH: Lung Health Study, NIH: Lung Tissue Research Consortium, NIH: SPIROMICS, Pittsburgh SCCOR

Inability to provide personal telephone contact number(s) and complete information for two additional contacts (next of kin, relative or close friend, not living with subject)

No place of permanent residence for the prior three months

Alpha-1 Antitrypsin deficiency

Regular use of an inhaled medication

Regular marijuana use (more than once per week)

HIV Positive

History of transplant of any organ

Active drug or alcohol dependence

History of employment in underground mining

Body mass index greater than 35

Temporary Exclusion Criteria

Subjects with the following may not have a study visit performed, but may be re-screened at a later time.

1. Pregnancy or suspected pregnancy. (These subjects will be re-scheduled for a visit at least three months after childbirth or after they are no longer suspected of pregnancy.)
2. Use of antibiotics for a respiratory infection within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the antibiotics.)
3. Use of systemic corticosteroids (new prescription or increased dose) for a respiratory disorder within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the steroids.)
4. Chest or abdominal surgery in the past three months. (These subjects will be rescheduled for a visit at least three months after surgery.)
5. Heart attack in the last three months. (These subjects will be rescheduled for a visit at least three months after the heart attack.)
6. Detached retina or eye surgery in the past three months. (These subjects will be re-scheduled for a visit at least three months after surgery.)
7. Hospitalization for any other heart problem in the past month. (These subjects will be rescheduled for a visit at least 4 weeks after hospital discharge.)

Subject Inclusion/Exclusion Criteria – Phase 2 Return Visit

Inclusion Criteria

Previously enrolled in COPDGene and meeting initial eligibility criteria at Visit 1.

Control subjects without a history of cigarette smoking (see criteria above).

Temporary Exclusion Criteria

Subjects with the following will be temporarily excluded but may be rescheduled at a later time:

1. Pregnancy or suspected pregnancy. (These subjects will be re-scheduled for a visit at least three months after childbirth or after they are no longer suspected of pregnancy.)
2. Use of antibiotics for a respiratory infection within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the antibiotics.)
3. Use of systemic corticosteroids (new prescription or increased dose) for a respiratory disorder within the past month. (These subjects will be rescheduled for a visit at least 4 weeks after completion of the steroids.)
4. Chest or abdominal surgery in the past three months. (These subjects will be rescheduled for a visit at least three months after surgery.)
5. Heart attack in the last three months. (These subjects will be rescheduled for a visit at least three months after the heart attack.)
6. Detached retina or eye surgery in the past three months. (These subjects will be re-scheduled for a visit at least three months after surgery.)
7. Hospitalization for any other heart problem in the past month. (These subjects will be rescheduled for a visit at least 4 weeks after hospital discharge.)

Rationale: All previously enrolled subjects from COPDGene are eligible for the study. Previous exclusions for lung cancer and other conditions are not a reason to exclude subjects from follow-up evaluation because these are outcomes of the disease to be assessed.

We will exclude patients with recent use of antibiotics and/or systemic steroids for a COPD exacerbation since such subjects may have an ongoing inflammatory pulmonary process related to an infection that will impair our ability to characterize and phenotype patients in their usual stable state.

We will exclude subjects who have contraindications to spirometry (exclusion criteria 4 – 7 above).

Subjects with exclusion criteria will be re-screened at a later date when they can be enrolled in Visit 2. The Administrative Core should be contacted regarding subjects who cannot complete all components of the study visit due to temporary exclusions or other medical conditions. The Administrative Core in consultation with the local clinical center director will determine which portions of a study visit can be completed. Those modifications will be considered if the subject is unlikely to be able to return at a later date for the study visit or unlikely to resolve medical issues that limit participation.

Phase 2

All Phase 1 subjects have provided consent with their initial study enrollment to be contacted again for further research studies. Subjects will be sent a letter advising them that funding has been provided for the second visit and informing them that they will be contacted by letter, email and/or phone to schedule an appointment for Visit 2. A telephone call will be made to each subject to schedule a date for his/her next study visit and ask them to bring the following to the

study visit: 1) all their current oral and inhaled medications, 2) names of any injectable study medications received on a regular basis, 3) social security number, 4) name, address and phone number of their personal physician, 5) name, address and phone of two other individuals likely to know the subject's whereabouts, at least one of whom is a relative not living with the subject, and 6) driver's license or other identification to assure they are the individual enrolled in COPDGene Phase 1.

At the Phase 2 study visit the subject will review the informed consent form for the second study visit and its study procedures. The subjects will have the opportunity to review the study documents in private, have questions answered and sign the informed consent. If necessary, subjects may review the informed consent with family members or their physicians. A HIPAA authorization or combined consent and HIPAA authorization will be reviewed and signed at the same time. Although subjects have signed a HIPAA authorization in Phase 1, a current authorization will be obtained to meet the requirements of hospitals and health care providers. Phase 2 data collection includes transfer of subject identifiers to the Data Coordinating Center (DCC) as described in the Central Data, Imaging, and Human Subject Contacts Protocol.

Mail Consent

Prior to the return visit, subjects may be contacted by the Clinical Center via mail to request additional consent and authorization. This additional consent and authorization will request that the subject allow transfer of identifiers from the Clinical Center to the Data Coordinating Center at National Jewish Health. This Mail Authorization provides permission for the Clinical Center to transmit the subject's personal information (social security number, address, secondary contact address) to the DCC. A Personal Contact Information Update form will be sent with the mail consent, asking subjects to update contact information, including secondary contacts and primary care physician. Additionally the subjects will be invited to sign an "Authorization to Release Protected Health Information" medical record release form.

This information will be used for central subject tracking, longitudinal follow up, vital status searching, and to obtain cost and utilization information on subjects from Medicare. The mail consent also allows the Clinical Center to seek perimortem medical records and informant interviews about the circumstances around the subject's death.

Visit 2 Options for Subjects Who Have Difficulty with a Visit to Clinical Center

In the event that a Phase 1 subject has moved away from their local Clinical Center and to an area closer to another participating COPDGene Clinical Center, the subject may elect to complete Visit 2 at this closer Center rather than traveling to their original Center. With the subject's verbal approval by phone, their new contact information will be provided from the Clinical Center where the subject had the first visit to the geographically proximate Clinical Center. The subject will sign a new Informed Consent Form and HIPAA with the coordinator at the new study location prior to their Phase 2 visit.

In the event that Phase 1 subject has financial hardship or has moved a considerable distance from their local Clinical Center, the COPDGene Administrative Core may consider providing transportation assistance to this subject for a return visit. The payment for this subject's return visit may include the actual reasonable cost of transportation, lodging, and meal expenses. Subject must provide receipts to Clinical Center for reimbursement. Use of this approach and funding for these expenses will be allocated on a case-by-case basis from the Administrative Core to the Clinical Center.

COPDGene subjects who are unable to come to a COPDGene Clinical Center to complete a Phase 2 visit due to illness, disease progression, or inability to be transported to the Clinical Center, will be given the option of having a research coordinator complete a partial study visit at the subject's place of residence. This home visit will include spirometry before and after albuterol administration, blood drawing, and completion of all study questionnaires.

Phase 1 subjects who are unable to come to a Clinical Center and are not willing or able to undergo a home visit will be asked if they would mind completing study questionnaires over the phone. If the subject agrees, this will be completed. If the subject is not willing or unable to complete study questionnaires over the phone they will be ask if they would complete a Limited Follow-Up Questionnaire and some or all of the regular study questionnaires. The Limited Follow-Up Questionnaire will be used to gather important longitudinal information on the subject related to respiratory symptoms, exacerbations, and comorbidities. A research coordinator will conduct this questionnaire with the subject by phone. These subjects may still participate in the bi-annual Longitudinal Follow-Up program by phone or Internet.

Phase 1 subjects who do not agree to a Phase 2 visit or to participate in Longitudinal Follow-Up will be asked if they would be willing to complete a Limited Follow-Up Questionnaire by phone. If the subject refuses to complete this questionnaire, their wishes will be respected.

Closed Clinical Center
Due to the longitudinal nature of the study, it is important to follow health and vital status on all of the COPDGene subjects. In the event that a clinical center is unable to perform follow up contacts or visits, the COPDGene Administrative Core will work as an agent for the Clinical Center or provide a contract clinical research organization to manage the follow-up subject visits and contacts per protocol. Subjects will be consented in Phase 2 to allow for a member of COPDGene Central Administration to contact them for follow up. The original clinical center must work with the Administrative core to facilitate efficient transfer of information and study management under the supervision of a local principal investigator and with the approval of the local IRB.

III. PHASE 2 STUDY PROCEDURES

Similar to the first visit, for the Phase 2 study visit there will be one to two visits for the majority of subjects depending on whether a separate visit is needed to schedule a chest CT scan. In some cases, subjects may have a third visit to re-collect information that does not meet quality control criteria. For example, if spirometry does not meet quality control criteria as judged by the Pulmonary Function Core, the subject may be contacted by phone to schedule an additional visit to repeat the test.

During the Phase 2 research study visit, the following procedures will be performed:

1. Informed consent will be obtained prior to any other study procedure. An updated HIPAA consent to obtain medical records to adjudicate the cause of death will be obtained. Subjects will be asked to provide a signed release of medical records to be used in the event of their death, to obtain an interview with a next of kin, secondary contact, or personal representative and with the treating physician. Hospital and physician records, related to the

events and illnesses associated with a participant's death, will also be obtained through the signed release of medical records. Subjects will be provided a memo to give to their physician(s) for this purpose.

2. Subjects will be asked to provide or update contact information of two secondary contacts with addresses and phone numbers, one of who is a next of kin or personal representative, and the other a relative or close friend, not living with the subject. The purpose of this information is to locate subjects who have moved to a different home and have a different address and/or phone number and ascertain vital status of the subject. The subjects will be asked to give consent to have their participation in the COPDGene study disclosed to these secondary contacts in the event that we are unable to contact them, and also disclosed to their next of kin, personal representative or family members in the event of their death. Subjects will be provided a memo to give to their secondary contacts for this purpose.
3. Prior to any other evaluations, an Enrollment/Safety Questionnaire will be administered to determine if potential subjects have a condition that would require deferring their study visit. They will be asked if they have a history of adverse events with the use of albuterol prior to administration of this inhaled bronchodilator. This safety assessment will also be used to ascertain potential cardiac disorders prior to albuterol administration and the walk test. If the questionnaire uncovers known or potential adverse effects of albuterol or potential cardiac disorders, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a six-minute walk test. This questionnaire is located on the protected study web site.
4. Updated contact information will be collected from the subjects. Name, home address, home phone number, cell phone, email address, date of birth and social security number will be collected or verified from the subject. The purpose of this information is to maintain contact with the subjects twice a year, and inform the subjects of other research studies and potential future COPDGene follow-up visits pending future funding. In order to perform central collection of mortality and cause of death, this information will be securely transmitted to the Data Coordinating Center at National Jewish Health, where it will be stored separately from the clinical, physiological, imaging, and genetic data. The transmittal of subjects' personal information to the DCC will be used for central subject tracking, longitudinal follow-up, death searching through the Social Security Death Master File (SSDMF) and to obtain cost and utilization information from the Centers for Medicare and Medicaid Services (CMS).
5. Physical Assessment will be performed on all subjects: A limited assessment will be performed including height, weight, arm span, waist circumference, blood pressure, heart rate, and room air oxygen saturation by pulse oximetry with the subject seated at rest. Blood pressure will be measured three times with the subjects seated and resting for at least five minutes. In subjects using supplemental oxygen, the oxygen will be withheld and subjects will breathe room air for 10 minutes prior to recording oxygen saturation. If resting oxygen saturation falls below 82% or the subject becomes dyspneic prior to 10 minutes without oxygen, oxygen therapy will be restarted as a safety precaution. If heart rate is less than 50 or greater than 110 or if the blood pressure is greater than 170/110, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a walk test.
6. Pre and post-bronchodilator spirometry will be performed on all subjects. The maneuvers are performed in a standardized manner. To obtain 3 acceptable measures, the technician

may ask the subject to perform up to 8 attempts. Spirometry is performed before and then repeated approximately twenty minutes following the administration of two puffs (180 mcg) of albuterol from a metered dose inhaler with a spacer. Spirometry will be performed with the subject in a seated position with a nose clip in place.

7. Diffusing capacity will be measured on all subjects after post-bronchodilator spirometry. The test will be performed according to American Thoracic Society/European Respiratory Society standards (8). In subjects using supplemental oxygen, oxygen will be removed and subjects will breathe room air for 10 minutes prior to the test. If resting saturation falls below 82% or the subject becomes dyspneic prior to 10 minutes without oxygen, oxygen therapy will be restarted as a safety precaution and the test will be performed without the ten-minute oxygen washout. To obtain 2 reproducible measures, the test may be repeated up to five times.

8. Standardized questionnaires will be completed by all subjects to assess respiratory symptoms, smoking history, and other medical history. These questionnaires include the St. George's Respiratory Questionnaire to assess health-related quality of life, the Medical Outcomes SF-36 to assess generic quality of life, and the COPD Assessment Test (CAT) to assess COPD severity. Questionnaires will be administered to obtain information about respiratory history, medical history, anxiety and depression (Hospital Anxiety and Depression Scale) (9), menstrual, pregnancy and female hormone medication history in women (Women's Questionnaire), current and previous residential history (Residential/ Occupational Questionnaire) to ascertain air pollution and environmental exposures, and occupational history. Subjects will be asked about access to regular medical care, access to health insurance and attitudes about medical care for their lung disease (Socioeconomic Questionnaire) to determine the impact of these factors on lung disease and exacerbations. If a participant is found to have cancer, determined by the Medical History Questionnaire, a Cancer Data Collection Questionnaire will be administered during the study visit to collect additional data on the cancer diagnosis. The subject may be invited to sign a tissue release addendum in order to provide formalin fixed paraffin embedded (FFPE) tissue blocks if there was a cancer diagnosis or lung tissue removed. An e-Cigarette questionnaire will be administered. Subjects will answer questions to assess amount of use and attitudes.

   Each patient will be asked to bring in all of their current medications in their original bottles. A list of current medications will be made at the local clinical center and transmitted to the Data Coordinating Center as a pdf document coded with the subject's study ID number. Oxygen use will also be recorded. A clinical coordinator will administer the Questionnaires. The results will be entered directly into the computer or recorded on paper forms to be input later by the coordinator. Completion of all questionnaires is expected to take between 90 and 120 minutes.

9. Blood will be drawn from all subjects for DNA (genetic and epigenetic studies), serum and plasma (for measurement of other proteins potentially related to COPD and other diseases) and a CBC (complete blood count). A total volume of approximately 50 ml of blood will be drawn for this study. A complete blood count will be performed at the Clinical Centers. The rest of the blood samples will be stored at the COPDGene Biological Repository at Brigham and Women's Hospital.

10. Six-Minute Walk Test will be performed in a standardized manner on all subjects to determine exercise capacity (11). Subjects will be asked questions to assure they do not

have significant or occult heart disease prior to the test to assure subject safety. Subjects who have significant cardiovascular disease may require evaluation by the local physician investigator prior to six-minute walk test. Immediately and one minute after the end of the six-minute walk test, a subject's heart rate and oxygen saturation will be measured using a pulse oximeter.

11. Chest CT Scans will be performed to assess emphysema and airway disease in all subjects. An inspiratory chest CT scan will be performed with a radiation dose of 200 mAs in order to provide thorough assessment of small airway wall thickness and emphysema. An expiratory CT acquisition will be performed using a fixed x-ray tube current of 50 mAs to assess for air trapping. As of June 2015, in 3753 subjects scanned for COPDGene Phase 2 using a 200 mAs inspiratory scan and a 50 mAs expiratory scan, the estimated median total effective dose was 8.2 mSv. An additional inspiratory chest CT scan will be performed using a low dose protocol aligned with current clinical practice that utilizes dose modulation. Dose modulation automatically adjusts x-ray exposure for each patient to achieve a consistent level of image quality. The reference mAs for this scan will be set such that an average sized subject (~75 kg) with a normal build would receive an x-ray exposure (CTDIvol) of 3.0 mGy (corresponding roughly to an effective dose of 1.5mSv). The scans will be processed using filtered back projection and iterative reconstruction where feasible. X-ray exposure will automatically be adjusted higher or lower for larger or smaller subjects. The maximum exposure for the dose modulation acquisition will be set at 3.0 mSv. Comparison (quantitatively and qualitatively) of the two inspiratory CT scans will be performed to allow generalization of the study results to the low dose scans being performed clinically nationwide and enable application to clinical practice. Pending IRB approval, all Clinical Centers will perform this additional low dose inspiratory scan (absent local technical or logistical issues).

    If the chest CT scans are done on the same day as the post-bronchodilator spirometry, the scan should be scheduled as soon as possible after albuterol. The time and date of the last bronchodilator prior to the CT scan will be captured. CT scans will be electronically transmitted to the Imaging Core at National Jewish Health by secure FTP site.

12. A urine pregnancy test will be performed on all premenopausal women prior to the chest CT scan to confirm that they are not pregnant.

13. Medical Record Review: medical records may be reviewed when available and with a signed release of records form documenting the subject's permission to obtain information about lung diseases, cancer, other medical conditions, and to determine cause of death, in the event of death.

Home Visit

If a COPDGene home visit is performed, it will include Home Visit Informed Consent and HIPAA administration, pre and post-bronchodilator spirometry, blood drawing, completion of all study questionnaires and procedures except six minute walk, diffusing capacity, and CT scan.

Visit 1 Study Procedures for Non Smoker Controls

Non-smoker controls enrolled during the Phase 2 study will be assessed for eligibility using the inclusion and exclusion criteria listed previously for the new non-smoker controls. For the study visit there will be one to two study visits for the majority of subjects depending on whether a separate visit is needed in order to schedule a chest CT scan. In some cases, subjects may have a

third visit to re-collect information that does not meet quality control criteria. For example, if spirometry does not meet quality control criteria as judged by the Pulmonary Function Core, the subject may be contacted by phone to schedule an additional visit to repeat the test.

During the Phase 2 research study visit, the following procedures will be performed:

1. Informed consent will be obtained prior to any other study procedure. An updated HIPAA consent to obtain medical records to adjudicate the cause of death will be obtained. Subjects will be asked to provide a signed release of medical records to be used in the event of their death, to obtain an interview with a next of kin, secondary contact, or personal representative and with the treating physician. Hospital and physician records, related to the events and illnesses associated with a participant's death, will also be obtained through the signed release of medical records. Subjects will be provided a memo to give to their physician(s) for this purpose.
2. Subjects will be asked to provide contact information of two secondary contacts with addresses and phone numbers, one of whom is a next of kin or personal representative, and the other a relative or close friend, not living with the subject. The purpose of this information is to locate subjects who have moved to a different address and/or phone number and ascertain vital status of the subject. The subjects will be asked to give consent to have their participation in the COPDGene study disclosed to these secondary contacts in the event that we are unable to contact them, and also disclosed to their next of kin, personal representative or family members in the event of their death. Subjects will be provided a memo to give to their secondary contacts for this purpose.
3. Prior to any other evaluations, an Enrollment/Safety Questionnaire will be administered to determine if potential subjects have a condition that would require deferring their study visit. They will be asked if they have a history of adverse events with the use of albuterol prior to administration of this inhaled bronchodilator. This safety assessment will also be used to ascertain potential cardiac disorders prior to albuterol administration and the walk test. If the questionnaire uncovers known or potential adverse effects of albuterol or potential cardiac disorders, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a six-minute walk test. This questionnaire is located on the protected study web site.
4. Updated contact information will be collected from the subjects. Name, home address, home phone number, cell phone, email address, date of birth and social security number will be collected or verified from the subject. The purpose of this information is to maintain contact with the subjects twice a year, and inform the subjects of other research studies and potential future COPDGene follow-up visits pending future funding. In order to perform central collection of mortality and cause of death, this information will be securely transmitted to the Data Coordinating Center at National Jewish Health, where it will be stored separately from the clinical, physiological, imaging, and genetic data. The transmittal of subject's personal information to the DCC will be used for central subject tracking, longitudinal follow-up, death searching through the Social Security Death Master File (SSDMF) and to obtain cost and utilization information from the Centers for Medicare and Medicaid Services (CMS).
5. Physical Assessment will be performed on all subjects: A limited assessment will be performed including height, weight, arm span, waist circumference, blood pressure, heart rate, and room air oxygen saturation by pulse oximetry with the subject seated at rest.

Blood pressure will be measured three times with the subjects seated and resting for at least five minutes. If heart rate is less than 50 or greater than 110 or if the blood pressure is greater than 170/110, a study physician will evaluate the subject to determine if it is safe to administer albuterol and perform a walk test.

6. Pre and post-bronchodilator spirometry will be performed on all subjects. The maneuvers are performed in a standardized manner. To obtain 3 acceptable measures, the technician may ask the subject to perform up to 8 attempts. Spirometry is performed before and then repeated approximately twenty minutes following the administration of two puffs (180 mcg) of albuterol from a metered dose inhaler with a spacer. Spirometry will be performed with the subject in a seated position with a nose clip in place.

7. Diffusing capacity will be measured on all subjects after post-bronchodilator spirometry. The test will be performed according to American Thoracic Society/European Respiratory Society standards (8). To obtain 2 reproducible measures, the test may be repeated up to five times.

8. Standardized questionnaires will be completed by all non-smoker control subjects to assess respiratory symptoms, occupational exposures, and other medical history. These questionnaires include a modified version of the American Thoracic Society/Division of Lung Diseases Respiratory Epidemiology Questionnaire to assess respiratory symptoms and St. George's Respiratory Questionnaire to assess health-related quality of life, the Medical Outcomes SF-36 to assess generic quality of life, and the COPD Assessment Test (CAT) to assess COPD severity. Questionnaires will be administered to obtain information about respiratory history, medical history, anxiety and depression (Hospital Anxiety and Depression Scale) (13), menstrual, pregnancy and female hormone medication history in women (Women's Questionnaire), current and previous residential history (modified Residential/Occupational Questionnaire) to ascertain air pollution and environmental exposures, and occupational history. Subjects will be asked about access to regular medical care, access to health insurance and attitudes about medical care for their lung disease (Socioeconomic Questionnaire) to determine the impact of these factors on lung disease and exacerbations. If a participant is found to have cancer, determined by the Medical History Questionnaire, a Cancer Data Collection Questionnaire will be administered during the study visit to collect additional data on the cancer diagnosis. The subject may be invited to sign a tissue release addendum in order to provide formalin fixed paraffin embedded (FFPE) tissue blocks if there was a cancer diagnosis or lung tissue removed. An e-Cigarette questionnaire will be administered. Subjects will answer questions to assess amount of use and attitudes. Each patient will be asked to bring in all of their current medications in their original bottles. A list of current medications will be made at the local clinical center and transmitted to the Data Coordinating Center as a pdf document coded with the subject's study ID number. A clinical coordinator will administer the Questionnaires. The results will be entered directly into the computer or recorded on paper forms to be input later by the coordinator. Completion of all questionnaires is expected to take between 90 and 120 minutes.

9. Blood will be drawn from all subjects for DNA (genetic and epigenetic studies), serum and plasma (for measurement of other proteins potentially related to COPD and other diseases), and a CBC (complete blood count). A total volume of approximately 50 ml of blood will be drawn for this study. A complete blood count will be performed at the Clinical Centers.

The rest of the blood samples will be stored at the COPDGene Biological Repository at Brigham and Women's Hospital.

10. Six-Minute Walk Test will be performed in a standardized manner on all subjects to determine exercise capacity (11). Subjects will be asked questions to assure they do not have significant or occult heart disease prior to the test to assure subject safety. Subjects who have significant cardiovascular disease may require evaluation by the local physician investigator prior to six-minute walk test. Immediately and one minute after the end of the six-minute walk test, a subject's heart rate and oxygen saturation will be measured using a pulse oximeter.

11. Chest CT Scans will be performed to assess emphysema and airway disease in all subjects. An inspiratory chest CT scan will be performed with a radiation dose of 200 mAs in order to provide thorough assessment of small airway wall thickness and emphysema. An expiratory CT acquisition will be performed using a fixed x-ray tube current of 50 mAs to assess for air trapping. As of June 2015, in 3753 subjects scanned for COPDGene Phase 2 using a 200 mAs inspiratory scan and a 50 mAs expiratory scan, the estimated median total effective dose was 8.2 mSv. An additional inspiratory chest CT scan will be performed using a low dose protocol aligned with current clinical practice that utilizes dose modulation. Dose modulation automatically adjusts x-ray exposure for each patient to achieve a consistent level of image quality. The reference mAs for this scan will be set such that an average sized subject (~75 kg) with a normal build would receive an x-ray exposure (CTDIvol) of 3.0 mGy (corresponding roughly to an effective dose of 1.5mSv). The scans will be processed using filtered back projection and iterative reconstruction where feasible. X-ray exposure will automatically be adjusted higher or lower for larger or smaller subjects. The maximum exposure for the dose modulation acquisition will be set at 3.0 mSv. Comparison (quantitatively and qualitatively) of the two inspiratory CT scans will be performed to allow generalization of the study results to the low dose scans being performed clinically nationwide and enable application to clinical practice. Pending IRB approval, all Clinical Centers will perform this additional low dose inspiratory scan (absent local technical or logistical issues).

    If the chest CT scans are done on the same day as the post-bronchodilator spirometry, the scan should be scheduled as soon as possible after albuterol. The time and date of the last bronchodilator prior to the CT scan will be captured. CT scans will be electronically transmitted to the Imaging Core at National Jewish Health by secure FTP site.

12. A urine pregnancy test will be performed on all premenopausal women prior to the chest CT scan to confirm that they are not pregnant.

13. Medical Record Review: medical records may be reviewed when available and with a signed release of records form documenting the subject's permission to obtain information about lung diseases, cancer, other medical conditions, and to determine cause of death, in the event of death.