Alpha-1 Carrier Genomics Study

Study Protocol and Statistical Analysis Plan

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ALPHA-1 CARRIER GENOMICS STUDY

ALPHA-1 PROTOCOL

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1. INTRODUCTION

Alpha-1 Antitrypsin Deficiency (AATD, Alpha-1) is an autosomal co-dominant genetic condition that predisposes to early onset chronic obstructive pulmonary disease (COPD) and liver disease. The *SERPINA1* gene instructs production of alpha-1 antitrypsin (AAT), a protease inhibitor that protects lung tissue from damage due to uninhibited neutrophil elastase. AAT is primarily produced in the liver. The wildtype *SERPINA1* gene encodes for normal AAT type M. Classic severe AATD results from the presence of two deficiency alleles. Numerous deficiency alleles have been reported and the homozygous state for the Z allele (PiZZ) is the most common severe deficiency type. The heterozygous PiMZ state results in a more mild deficiency in serum AAT and thus lower disease risk in most individuals. However, there exists heterogeneity of clinical presentation in most populations of AATD and genetic variation in the Alpha-1 gene has been incompletely studied. Rare gene alterations that predispose to COPD risks of classic AATD in individuals without classic homozygous deficiency genotype have not been studied and are important in understanding, testing and treating at-risk populations.

Demeo et al. showed this heterogeneity between age and smoking status in a study of PiZZ sibling pairs [1]. More recently, Molloy has demonstrated worse lung function in PiMZ individuals who have a history of smoking compared to non-smokers [2]. In this study of non-index PiMM and PiMZ siblings, there was also found worse lung function associated with PiMZ non-smoking genotypes between MM and MZ subjects. Group mean lung function differences can hide the clinical observation that some PiMZ individuals present at young age with classic panlobular emphysema similar to PiZZ AATD. Mechanisms to detect the genetic signatures that are associated with these advanced clinical phenotypes would allow improved diagnostics and therapeutics for these individuals' lung disease.

Genetic variation in the Alpha-1 gene has been incompletely studied. While historical texts suggest ~100 different SERPINA1 alleles, modern genetic databases remain largely proprietary to each lab but > 500 AAT alleles are now estimated. This biases against publication of new variants. Of some interest is the ability to sequence upstream and downstream from the coding sequence of *SERPINA1* which has allowed detection of significant deletions of major portions of the gene. With this background **we hypothesize that *SERPINA1* gene sequencing performed through the DNA1 testing platform will find important sequence variations in individuals who have COPD compared to age, race, sex, AAT level and smoking status matched individuals who do not have COPD in the PiMZ ACT cohort.**

The Alpha Coded Testing (ACT) Study is an established and confidential home genetic testing study for alpha-1 antitrypsin deficiency (AATD). Two primary populations choose to test through ACT. This is a preferred program for individuals in the US to test family members of those who have been found severely deficient. In addition, this test is used by individuals with COPD who choose to test in a confidential manner. The ACT Study is sponsored and endorsed by the Alpha-1 Foundation. As a result of the demographics, 27% of the ACT cohort adults who complete testing are found to have one Z allele (N=4153). The serum levels estimated from these blood spot cards are non-normally distributed with significant variation.

ACT participants with heterozygous genotype MZ will be invited to participate in this matched-control study. In the absence of spirometry in ACT, the validated COPD severity score[3] has been used to define

the presence and grade the severity of COPD from questionnaires. The primary outcome of this study will capture the number and features of *SERPINA1* variants between the two groups and allow correlation to COPD presence and severity.

Specific Aims

1.1 To identify rare *SERPINA1* sequence variants that correlate with presence and severity of COPD in MZ carriers of Alpha-1 antitrypsin deficiency.

1.2 To determine the number of individuals who can perform a home test with the DNA1 kit with sufficient blood for analysis.

1.3 To correlate COPD severity scores with specific *SERPINA1* variants.

This matched-pair pilot study will allow for the number of likely significant gene mutations between COPD and matched control groups to be compared using a paired T-test. Descriptive analysis will link all areas of *SERPINA1* genomic variation with presence and severity of COPD.

2. BACKGROUND

2.1 Literature and Previous Studies

Alpha-1 Antitrypsin Deficiency (AATD, Alpha-1) is the best established genetic risk factor for chronic obstructive pulmonary disease (COPD) and liver disease. COPD represents a major health burden, with nearly 12.6 million individuals in the United States diagnosed. COPD is a significant cause of morbidity, mortality, and related social and financial burden. Understanding heritable and environmental risk factors for COPD is important to guide targeted risk reduction recommendations, detection, and intervention.

AATD-related COPD is phenotypically indistinguishable from idiopathic COPD and approximately 1-3% of COPD is related to severe homozygous AAT deficiency. AATD is an autosomal co-dominantly inherited susceptibility. The *SERPINA1* gene on chromosome 14 encodes production of alpha-1 antitrypsin (AAT), a protease inhibitor that is primarily produced in the liver. The wildtype gene produces the normal AAT protein M, which in circulation is protective to the lungs through inhibition of neutrophil elastase. Two specific *SERPINA1* mutations account for approximately 95% of Alpha-1 deficiency alleles, though over 500 deficiency alleles are now estimated. These common mutations produce the common abnormal AAT proteins S and Z, respectively, which result in corresponding serum deficiency of AAT. Uninhibited neutrophil elastase predisposes to COPD, while the Z protein specifically aggregates in the liver and raises risk for liver disease. Other rare mutations in the *SERPINA1* gene also result in abnormal AAT protein production, leading to serum deficiency or nonfunctional AAT and similar disease risks.

Severe classic AATD occurs in the presence of two abnormal alleles (e.g. ZZ, SZ), where wildtype-deficiency heterozygotes (e.g. MZ, MS) are at lower disease risk; however, there exists heterogeneity of clinical presentation in most populations of AATD and understanding other genetic and environmental modifiers of risk in each group is important. Because AAT production is allele-dependent, heterozygotes, or carriers, have intermediate levels of circulating AAT with values lower than that of homozygous wildtype genotype MM but higher than levels of classic deficiency. The MM genotype corresponds with serum AAT levels of 19.0-59.7 uM, while classic severely affected ZZ individuals have serum levels of less than 11 uM. MZ heterozygote serum levels range from 11 to 48 uM.

Although AAT concentration greater than 11 uM has conventionally been believed to be sufficient, recent data suggest increased susceptibility to COPD among MZ individuals in the presence of additional risk factors. Malloy, et al (2014) found that MZ individuals who smoke are more susceptible to COPD than a control group of matched wildtype MM smokers. In the absence of this other major risk factor studied (smoking), COPD risk was not significantly different between MZ and MM groups. Smoking is the leading environmental modifier of the onset of COPD symptoms in populations with and without AATD.

Some MZ individuals present at a young age with classic panlobular emphysema similar to that which is common ZZ individuals and uncommon in the wildtype population. Group mean lung function differences can hide the clinical observation that some PiMZ individuals appear to be particularly susceptible to an AAT-related phenotype. Genotype is traditionally established by several of paradigms discussed below, yet genetic variation in the Alpha-1 gene has been incompletely studied. Rare gene alterations that predispose to COPD risks of classic AATD in individuals without classic homozygous deficiency genotype have not been studied. Such variants may occur in traditionally untested regions of the *SERPINA1* gene and serve as an important genetic modifier of risk in MZ individuals.

Defining both genetic and environmental susceptibility factors is important in understanding, testing and treating at-risk populations. Alpha-1 outcomes are improved in the contexts of presymptomatic risk determination and prompt diagnosis in the presence of clinical symptoms. People who receive presymptomatic genetic testing results consistent with AATD are less likely to adopt cigarette smoking and more likely to quit. Further, occupational choices may be made to minimize exposure to inhaled particulates and early medical care may be sought at the onset of AAT-related COPD symptoms. Among COPD-symptomatic individuals, recognition of underlying AATD is critical, as a primary treatment exists to prevent or delay progression of symptoms in this COPD subpopulation. Augmentation therapy involves infusion of purified human AAT protein and circulating serum AAT levels within normal limits can thus be attained.

Several common AAT testing paradigms exist, including protease inhibitor typing by isoelectric focusing (PI typing), serum quantification of AAT, *SERPINA1* genotyping, and *SERPINA1* next generation sequencing. Pi typing and genotyping can detect the common deficiency protein types and genotypes, respectively. These methods ascertain the majority of carriers and classically affected individuals and correctly assign genotype in most cases. Serum AAT quantification does not definitively determine genotype; however, an unusually low AAT level when only one or no Pi or genotype abnormalities are detected indicates the likely presence of a rare or null allele. Next generation sequencing is a high-throughput and scalable sequencing platform able to clarify genomic sequence of many rare variants within coding regions of the *SERPINA1* gene. A majority of carriers and severely deficient individuals have not had any sequencing test performed. Next generation sequencing in these populations may reveal previously undetected but significant risk variants for Alpha-1.

The Alpha Coded Testing (ACT) study is an established and confidential home genetic testing study for Alpha-1. Over 4,000 MZ individuals have been identified through combined genotyping and AAT quantification. Individuals in ACT provide COPD data and indicate that they are willing to be contacted for possible participation in other research. These individuals have not had *SERPINA1* sequencing so rare genomic variants that may exist in this cohort are previously untested. *SERPINA1* next generation sequencing in MZ individuals with COPD and a control group of matched MZ individuals without COPD will be conducted to determine whether rare *SERPINA1* genetic signatures are biomarkers for presence and severity of MZ-associated COPD.

2.2 Rationale for the Study

Since it is largely unknown why some PiMZ individuals develop COPD similar to that of classic PiZZ Alpha-1 and which is rare in the general Pi MM population, we propose to study *SERPINA1* gene sequences in MZ individuals with and without COPD. Smoking has recently been found to be a significant gene-by-environment modifier of COPD risk. COPD and Alpha-1 are both multifactorial conditions, where multiple environmental and multiple genomic factors likely influence individual risk and disease course. Identifying both genetic and environmental contributors is important for improving Alpha-1 knowledge, recommendations and outcomes. This study will look for previously unknown gene-by-gene correlates as possible risk modifiers for COPD in the PiMZ population. Should such susceptibility biomarkers be determined, future screening recommendations may include gene sequencing as a primary screening or diagnostic test, or for such testing to be incorporated into personalized clinical care and management.

2.3 Primary Hypothesis

The overarching hypothesis of the MZ Carrier Genomics study is that *SERPINA1* gene sequencing performed through the DNA1 testing platform will find important sequence variations in individuals who

have COPD compared to age, race, sex and smoking status matched individuals who do not have COPD in the PiMZ ACT cohort.

3. STUDY OBJECTIVES

3.1 Primary Aim:

The number of individuals with additional *SERPINA1* mutations in the PiMZ COPD cohort will be compared to the number of individuals with additional *SERPINA1* mutations in the PiMZ cohort without COPD.

The primary analysis will determine whether *SERPINA1* genetic signatures detected by sequencing correlate with presence of COPD in PiMZ individuals.

3.2 Secondary Aims:

3.2.1 To determine the number of individuals who can perform a home test with the DNA1 kit with sufficient blood for analysis. Among people who desire to test, the portion of people able to successfully obtain a sufficient sample in the home setting using a DNA1 test kit will be determined.

3.2.2 To correlate COPD severity scores to specific *SERPINA1* variants.

3.3 To invite participation in the MUSC Alpha-1 Biorepository Participants will be invited to donate to the MUSC Alpha-1 biorepository by submitting samples in provided PAXgene DNA, PAXgene RNA and EDTA plasma tubes. Copies of spirometry will be requested and COPD questionnaire data linked. These biosamples will be linked to the sequence data for longitudinal transcriptional and proteomic data. Biologic samples are stored for future use with permission for study of Alpha-1, genomics and epigenetic factors. A separate informed consent for biorepository participation will be provided to interested individuals (Pro00039387).

4. RATIONALE FOR THE STUDY

4.1 This study seeks to identify sequence changes that predict COPD in MZ individuals.

It is unknown why some PiMZ individuals present at early ages with classic panlobar emphysema similar to that seen in classic PiZZ AATD. Previously untested regions of the *SERPINA1* gene may contain sequence alterations that predict this phenotype.

4.2 Invited participants

Participants tested through the ACT study have already been genotyped, AAT level tested, and reported presence or absence of COPD and demographic information. Classic homozygous AATD has been ruled out. Additionally, these individuals have consented to be contacted for possible participation in Alpha-1 and/or genetic testing-related studies. The MZ cohort with the lower quartile of AAT level is selected as the most reasonable first population for a study of this nature that seeks to identify whether previously untested *SERPINA1* variations correlate with COPD. MZ individuals in the lower quartile of level report the highest MZ incidence of COPD, and this cohort of MZs contains sufficient numbers to obtain matched pairs of COPD-affected and control individuals from this target group. Based on findings of this pilot study, studies of this nature may be expanded to include matched pairs from other MZ-level cohorts and other COPD populations in the future. The 25% of the MZ cohort with lowest levels will have A1-PI values from 11 to approximately 16 uM with the median MZ value equal 19.6 uM.

4.3 Use of the DNA1 kit

CSL Behring partnered with Biocerna LLC in 2013 to offer the DNA1 Advanced Alpha-1 Screening (DNA1) test, the only comprehensive Alpha-1 test available that identifies known and unknown clinically relevant genetic variants related to AATD. CSL Behring and has agreed to supply 150 DNA1 test kits for blood collection and perform testing on all returned participant samples in the Biocerna lab in conjunction with this study. Using the DNA1 test kit involves performing a finger stick in the home setting to obtain

spots of blood that are used for testing. Testing includes: clinical chemistry (a blood spot assay to test AAT levels, as well as a C-reactive protein (CRP) to provide full clinical analysis), targeted genotyping (identifies all known, clinically relevant variants, including S, Z, and F), next generation sequencing (sequencing of the *SERPINA1* gene to identify unknown variants), and isoelectric focusing (Results compared to genetic results as a confirmatory step). The next generation sequencing includes coverage of the promoter region, exons and intronic splice sites. The variety of genomic variations detected by sequencing of the *SERPINA1* gene will likely be large. Biocerna will define if they believe mutations are likely to be in functional regions of the gene, independent of knowing if the patient has COPD or not.

4.4 Administering COPD severity assessment

The St. George's Respiratory Questionnaire (SGRQ) is a COPD assessment that has been shown to correlate well with established measures of symptom level and disease activity. Presence and severity of COPD can thus be assigned for each participant. The COPD severity score collected previously through ACT study participation will be again administered as part of this study. Thus interval reports will be available as a proxy for disease progression for those who may not have spirometry available. Spirometry reports will also be requested and collected, where possible. The COPD severity score is a validated questionnaire-based instrument that is internally consistent, reliable, and appears to capture a broad range of disease severity. The COPD severity score survey may be administered by telephone or as a written questionnaire. COPD data will allow for correlation of COPD presence and severity with genomic findings.

Questionnaires will be formulated in REDCap, with hard copies available for mailing to participants who lack internet access or literacy. Following signed informed consent and HIPAA forms, participants will either be mailed questionnaires to complete and return or will be emailed a secure link for access to REDCap questionnaires, including the SGRQ, COPD severity score and demographic surveys. Any paper survey documents will be digitized, reviewed by a team member to confirm that all pages are present and legible, facing the appropriate direction and are an exact copy of the paper forms. Each page will be individually labeled with the participant's study ID and the question item responses provided will be entered by a trained study coordinator under the participant's ID in the electronic database. The original paper documents will be kept in a locked file cabinet in the Alpha-1 office.

4.5 Invitation to the biorepository

Participants will be asked to check Yes or No on this study's consent form regarding their interest in donating blood to the MUSC Alpha-1 Biorepository. Interested participants will be presented with the separate Biorepository informed consent (Pro39387). Upon providing consent participants who are not on-site at MUSC will be mailed PAXgene DNA, PAXgene RNA, and EDTA plasma tubes that require collection at a physician's office. Overnight return mailing and processing charges are included in study funding. The purpose of the Biorepository is to foster future biological research related to Alpha-1 and genomics. Interest in Biorepository participation does not impact participation in this study.

5. STUDY DESIGN AND PROCEDURES

5.1 Overview

The MZ Alpha-1 Carrier Genomics study is a pilot study that will enroll up to 150 MZ individuals, with a goal of attaining 50 matched pairs. COPD+ and COPD- individuals will be matched on age, sex, race and smoking history. Any consented individuals who do not have a match in the study cohort will be allowed to continue with participation. Their samples will be analyzed as unmatched COPD + or – samples, which will be accounted for statistically and discussed in study reports.

Presence and severity of COPD is assessed by a COPD severity score for each registrant in the ACT database. We will additionally ask as part of this study that current COPD questionnaires and demographic surveys be completed, and that patients submit previous lung function testing if this has been done. Participants will be mailed a DNA1 test kit to obtain and return a blood sample by finger stick for the purpose of *SERPINA1* gene sequencing. Gene sequencing at Biocerna LLC will identify, if present, genomic signatures that may correlate with COPD in this cohort. Biocerna LLC will determine likely pathogenicity of sequence variants detected blinded to knowing COPD-status of the patient tested. Statistical analysis will correlate genetic findings with COPD presence and severity. Participants will receive their results by mail. Participants will also be invited to contribute additional samples to the MUSC Alpha-1 Biorepository.

5.2 Participant Recruitment and Selection

There are over 370 individuals in the ACT MZ cohort with AAT levels below the 25th percentile who have previously provided COPD severity scores. We propose to invite these 370+ individuals to participate. Potential participants will be invited by research staff initially by telephone call, though subsequent contact may occur via alternative methods, including but not limited to email or home mailing per ACT protocol policy procedures. Eligible individuals who did not test through ACT and are able to provide documentation of their MZ and lower quartile level results will also be accepted into this study. The study personnel contacting the participant will describe the nature of the study and details of participation, including that questionnaire based responses, spirometry if available, and a blood sample by finger stick for genetic testing will be requested. Once participants are enrolled (signed informed consent and HIPAA), an email will be sent containing a secure link for recipients to follow to complete the questionnaires on REDCap. If needed, paper questionnaires may be mailed. Test kits will be mailed following receipt of completed questionnaires.

All participants may make a written decision separate from primary study consent and participation whether to allow their clinical and genomic data linked to personal identifiers to be stored in the MUSC Alpha-1 Biorepository indefinitely after the conclusion of this study. No personally identifying information will be published and personal information obtained and stored will remain confidential to the extent possible within State and Federal law.

5.3 Inclusion/Exclusion

5.3.1 Inclusion Criteria

1. Signed informed consent

2. PiMZ individuals who fall into the lower quartile of AAT levels.

5.3.2 Exclusion Criteria

1. Age <18 years

2. Known homozygous or compound heterozygous classic severe AATD (e.g. PiSZ, ZZ, Znull)

5.3.3. Conditional Exclusions

1. Incomplete return of study materials. Those who consent but fail to both complete questionnaires and return a blood sample may be excluded from analysis.

5.4 Consent

DoxyMe
We will telephone potential candidates for this study who are identified in the ACT database. A telephone script will identify the research coordinator and establish a time convenient for DoxyMe consent to begin.

Consent will review in detail the nature of the Alpha-1 Carrier Genomics Study, as well as potential risks and benefits of participation and the option to not participate.

Informed consent and signature of HIPAA will take place via DoxyMe, a free and HIPAA compliant telemedicine service. While the consenting research personnel and the potential participant are connected by video and voice on the DoxyMe server, the informed consent will be electronically sent to the participant via this platform for real time signature and return. The participant will have the informed consent and HIPPA form reviewed by the trained study coordinator. Adequate time will be given for each potential enrollee to read, understand and ask questions about the research study. Once the consent form is signed, dated and electronically returned via DoxyMe, the consenting coordinator will sign and date it, as well. At this point the participant is enrolled. The completed forms are then submitted to and stored in the electronic database established for the study. Participants will be encouraged to retain their copy for their records. Note that there is no paper copy of this consent form.

If necessary, paper consents may be mailed, with the consenting process taking place by telephone. In this event, following adequate opportunity to ask questions willing participants will sign the consent and HIPAA forms and return them to the consenting member of the research team by fax or mail. At the same time, the consenting member of the research team will also sign and date a copy of both the consent and HIPAA forms. Once the participant's signed forms are received, they will be stapled together with those signed by the consenting team member and a note entered at the bottom of the patient's consent linking the files. At this point the participant is enrolled. The complete paperwork is then scanned as a pdf and submitted to the electronic database established for the study. Participants will be encouraged to retain their copy for their records.

All pages of any paper consents will be digitized, reviewed by a team member to confirm that all pages are present and legible, facing the appropriate direction and are an exact copy of the paper consent. Each consent will be individually labeled with the participant's study ID. The consents will be saved in a secure server and indexed for immediate access by authorized study team members. Certification that the pdf consent is identical to the paper copy will be added as initials and date in adobe acrobat. After certification has been added the file will be locked and saved. The original paper consents will be kept in a locked file cabinet in the Alpha-1 office.

5.5 Withdrawal

A participant may withdraw from the study at any time for any reason. At the time of withdrawal the participant may 1) decline to provide any more data or specimens to the study but allow use of previously collected data and/or specimens, 2) request withdrawal of all his/her data from study databases and request that any stored samples be destroyed, or 3) withdraw some portion of the data collected (i.e. participants may withdraw specimens but not questionnaire data or vice versa). Genomic sequencing results in extensive genetic data, including genetic sequence that cannot be unknown. Laboratories often retain records of all sequence data that results from testing in their facility. Participants may not be able to recover all records of their genomic sequence; however, the laboratory sequence data will be linked only to study number in their records and no personally identifying information will be linked. Participants may specify that they wish for data that results from sequencing of their assigned study number to be omitted from research analysis or use; however, such data may not cease to exist upon withdrawal.

If a participant chooses to withdraw the research coordinator will determine the disposition of the participant's study data and provide the participant with any clinically relevant study results, or establish how the participant would like to be contacted if relevant results become available in the future.

5.6. Study Schedule

1. Following IRB approval and establishment of CSL contracts study coordinators will contact lower quartile MZ individuals by telephone to invite them to participate and schedule informed consent. Follow-up calls may be made to boost initial recruitment and matching.

2. For each informed consent and questionnaire set received, a barcoded DNA1 test kit will be mailed with instructions for obtaining and returning the required sample. Postage paid return mailing supplies will be provided.

3. The samples returned will go by US Postal Service directly to the Biocerna lab for testing. Biocerna will have the sample and barcode only (no personal health information), while all survey items and identifiers are collected and stored for linkage at MUSC.

4. MUSC will perform matching of age, sex, and smoking-status COPD-affected and control MZ participants.

5. Biocerna LLC will perform *SERPINA1* sequencing on samples sent by participants and provide laboratory interpretation of likely pathogenicity to study personnel.

6. Study personnel will link the results to the participant and mail results to the participant. The results will include a study interpretation of results and recommendations for accessing appropriate resources.

7.

8. Statistical analysis of all matched pairs using paired T-test and descriptive analysis will allow for conclusions of this pilot study to be made. Participants for whom no match is available in the study cohort will be included in an uneven COPD+ / - arm analysis and aggregate study reporting.

9. The study will be complete in approximately 2 years, with subsequent publication of results, including an abstract for the American Thoracic Society annual meeting in May 2016.

5.7.1 Blood Samples

Safety and Processing: Samples will be received by the CLIA certified Biocerna Laboratory in Gaithersburg, MD where safety and sample processing will occur per their usual protocols. Biocerna runs the DNA1 analysis on a clinical and commercial basis and has necessary and proper protocols in place to receive and handle specimens of this study.

5.7.2 Sequencing/ Integrated Genomics

Genetic sequencing is used to determine the order of nucleotides within a gene. State of the art next generation sequencing methods will be applied to detect sequence variants in all regions of the *SERPINA1* gene, including regions upstream and downstream of coding regions of the gene, promoters and regulatory regions. Such testing is more thorough than that which is traditionally performed on a clinical basis yet the role of various mutations within and around the SERPINA1 coding region is potentially important in pathogenicity. Genomic interpretation software and models will be used by Biocerna to assign likely pathogenicity of genomic findings blinded to knowing whether the individual who provided the sample has COPD.

5.8 Questionnaires

St. George's Respiratory Questionnaire (SGRQ)

This biomarker for COPD quantifies a patient reported outcome.

Repeat COPD Severity Score

This assesses clinical severity of COPD. Interval assessment allows for disease progression to be inferred.

Demographic questions not already in ACT

Demographic questions to ensure fidelity of matching will be asked. See corresponding questionnaire.

5.9 Ethical Considerations

5.9.1 Human Subjects' Protections and Risk

DNA1 test kit finger stick

Finger stick is a common method to obtain blood spots for testing. Blood obtained by finger stick involves puncture of the skin with a needle that can cause pain. Very rarely, infections can occur at the site of puncture. Proper safety instructions including proper disinfection of the puncture site prior to needle stick are provided with each test kit. The aforementioned risks are described in the informed consent.

Genetic Testing

SERPINA1 genetic testing risks include the possibility of identifying previously unknown genetic risk for COPD or identifying a genetic change for which the significance is unknown. Testing and results of testing may be associated with feelings of anxiety and uncertainty. Because genetic variants are often shared by family members, testing may inadvertently reveal risk to relatives. The aforementioned risks, as well as potential benefits of knowing genetic information, are described in the informed consent. Study personnel are available to answer questions participants may have prior to or during participation. The Alpha-1 Foundation, under direction of PI Charlie Strange and co-investigator Kim Brown, offers free genetic counseling services for Alpha-1. Genetic counseling may help people understand medical and familial implications of *SERPINA1* results and assist in psychosocial adaptation. The phone number for free genetic counseling service is provided on all results.

Confidentiality

There is a risk to confidentiality. Source data will be kept electronically under password protected systems. Questionnaires or paper documents will be kept in secure areas. Data transmission will be encrypted with a 128-bit VPN approach. Biocerna and MUSC systems are designed to be HIPAA compliant. Collection of DNA and RNA can lead to identification of patients although there is no attempt to do so in this project. Any loss of confidentiality from any participant will be reported to the IRB.

All study personnel are certified in the ethical conduct of human biomedical and genetics research and HIPAA information security. Proof of training will be collected from all study personnel.

Longer descriptions of human subjects protections are provided in the informed consent.

5.9.2 Adverse Events

Procedures for Adverse Events

Minimal risk for adverse events exists related to participation in this study. Pain and bruising may occur at the site of a needle stick; rarely, infections can occur at the site of puncture. In the event that a study coordinator is notified of an adverse event by phone, the coordinator will consult the MD PI to reasonably assess whether the participant warrants medical attention and advise as such. Any adverse event will be reported within one week of notification of event to the IRB. Study coordinators will comply with local regulations and policies when notifying the institutional IRB.

Procedures for Serious Adverse Events

The FDA (2009) defines a serious adverse event as an adverse event that: results in the participant's death, is life-threatening, results in hospitalization (initial or prolonged), results in significant, persistent, or permanent change, impairment, damage, disruption, or disability in the participant's body function/structure, physical activities or quality of life, results in a congenital anomaly, or requires intervention to prevent permanent impairment or damage.

Risk for serious adverse events is not anticipated.

Measures to Protect the Participant

Instructions for disinfecting the puncture site and safely performing a finger stick will be included with each DNA1 test kit. Further, a link to the YouTube instructional video that details safe and effective sample ascertainment will be provided.

6. STATISTICAL ANALYSIS

6.1 Primary Aim: The number of likely significant gene mutations between COPD and matched control groups will be compared using a paired T-test

The variety of mutations in non-coding and coding regions of the *SERPINA1* gene will be large. Therefore, Biocerna will define if they believe mutations are likely to be in functional regions of the gene, independent of knowing if the patient has COPD or is a matched control. The number of likely significant gene mutations between COPD and matched control groups will be compared using a paired T-test.

6.1.1 The nature of mutations identified will be correlated with COPD severity.

Descriptive analysis at the genomic level will follow to link all areas of genomic variation with severity of COPD. Functional gene expression and likely pathogenicity as determined in Biocerna interpretation will be related to established COPD phenotypes using linear models.

6.2.2 The portion of individuals who return a DNA1 test kit with sufficient blood sample for testing will be quantified for assessment of home testing feasibility.

6.2 Sample Size

There is not a power analysis associated with this pilot study.

The goal is to analyze 50 matched pairs, or 100 total samples. This study will enroll up to 150 individuals in order to have at least 100 samples returned to the lab for testing, as there are some people who consent but never return their test kit. Enrolling up to 150 allows better chances of finding 50 matched pairs. All samples that are received by the lab will be analyzed. If we end up with uneven numbers in the COPD+ and COPD- arms, more than 100 samples analyzed, or have participants without a match, we will account for this in the statistical analysis and reporting.

7. DATA HANDLING

7.1 Web Data Entry

Data entry is performed via REDCap and an encrypted, password protected database developed at MUSC for the purpose of clinical research. Results of pulmonary function tests, where provided, are entered by research coordinators and source documents saved in secured and locked areas according to Good Clinical Practice.

7.2 Integration with Biorepository

This study invites participation to the MUSC Alpha-1 Biorepository. Trained MUSC Alpha-1 research staff maintain logs of all biosamples and their distribution. All genomic sequence data are analyzed for variants associated with the phenotype of interest (AATD). RNA, mRNA, and proteomic data as available are linked with samples and corresponding clinical phenotype for the purpose of building longitudinal transcriptional and proteomic data. Biosamples are stored for future use with permission for study of Alpha-1 and related genomic and epigenetic factors. Sample use and regulations are specified in the Biorepository IRB Protocol (Pro00039387).

8. STUDY MONITORING

8.1 Data Fidelity and Quality Control

The clinical and biospecimen output data linked only to a study ID will be archived and stored in the corresponding secure database for a period of no less than 5 years after conclusion of the study. Data is password protected and secured by physical and firewall protected means.

8.2 Biospecimen Quality Control

Samples sent to CLIA certified Biocerna Lab are kept per their independent secure and certified sample processing protocols.

9. STUDY ADMINISTRATION

Charlie Strange, MD, oversees study administration and study personnel, including co-investigator and other trained research personnel who will be responsible for coordination and execution of this study. Under the auspices of Dr. Strange and institutional regulatory bodies study staff will coordinate meetings, conference calls, and web-conferences as needed; determine and delegate needs for administrative support (e.g. scheduling, minutes) for committees and subcommittees; ensure fidelity of all data management activities, including form design, regular reports, reimbursing, including contracts, identifying milestones for payment, and generating invoices and payments, maintaining directories and distribution lists, preparing reports, creating recruitment materials and assisting with recruitment, creating and updating the Manual of Operations, training and certification of study personnel, organizing in-person meetings, and coordinating and overseeing the publication process.

10. REFERENCES

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