



US 20250257403A1

(19) **United States**

(12) **Patent Application Publication**  
**WANG et al.**

(10) **Pub. No.: US 2025/0257403 A1**

(43) **Pub. Date: Aug. 14, 2025**

(54) **METHOD TO DETECT ABNORMAL  
ALPHA-1 ANTITRYPSIN DEFICIENCY  
(AATD) GENOTYPES**

**Related U.S. Application Data**

(60) Provisional application No. 63/341,300, filed on May 12, 2022.

(71) Applicant: **NATIONAL JEWISH HEALTH,**  
Denver, CO (US)

**Publication Classification**

(72) Inventors: **Yongbao WANG**, Denver, CO (US);  
**Iara MACHADO**, Denver, CO (US);  
**Ron HARBECK**, Denver, CO (US);  
**Robert SANDHAUS**, Denver, CO  
(US); **Claire COESHOTT**, Denver, CO  
(US)

(51) **Int. Cl.**

**C12Q 1/6883** (2018.01)

**C12Q 1/686** (2018.01)

**C12Q 1/6872** (2018.01)

(52) **U.S. Cl.**

CPC ..... **C12Q 1/6883** (2013.01); **C12Q 1/686**  
(2013.01); **C12Q 1/6872** (2013.01); **C12Q**  
**2600/156** (2013.01)

(21) Appl. No.: **18/864,402**

(22) PCT Filed: **May 12, 2023**

(86) PCT No.: **PCT/US2023/066932**

§ 371 (c)(1),

(2) Date: **Nov. 8, 2024**

(57)

**ABSTRACT**

Disclosed herein are methods and kits for detecting abnormal alpha-1 antitrypsin deficiency (AATD) genotypes.

**Specification includes a Sequence Listing.**

## METHOD TO DETECT ABNORMAL ALPHA-1 ANTITRYPSIN DEFICIENCY (AATD) GENOTYPES

### CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 63/341,300, filed May 12, 2022. The entire disclosure of U.S. Provisional Patent Application No. 63/341,300 is incorporated herein by reference.

### REFERENCE TO SEQUENCE LISTING

**[0002]** This application contains a Sequence Listing submitted as an electronic XML file named “2879-239-PCT.xml”, having a size of 11,000 bytes, and created on May 12, 2023. The information contained in this electronic XML file is hereby incorporated by reference in its entirety pursuant to 37 CFR § 1.52(e)(5).

### BACKGROUND

**[0003]** Alpha-1 antitrypsin deficiency (AATD) is a common, but under-diagnosed, inherited disease caused by specific serpin family A member 1 (SERPINA1) gene mutations. Genetic changes can lead to low or abnormal alpha-1 antitrypsin protein (AAT, also referred to as A1AT) production in liver cells, with resultant liver or lung damage. Current clinical diagnostic tests include measuring AAT protein levels, detecting deficient protein alleles by isoelectric focusing gel (IEF), genetic polymerase chain reaction (PCR) testing for major S and Z allele mutations as well as the normal allele, M, and whole gene sequencing. IEF is a labor-intensive assay and results can be hard to interpret and most PCR based genotyping assays only detect two major single nucleotide polymorphism (SNPs). Thus, there is a need for a more accessible genetic front-line test to cover more mutations which allows for broader screening for lung and liver diseases in patients.

### SUMMARY

**[0004]** One embodiment relates to a method to detect an abnormal alpha-1 antitrypsin deficiency (AATD) genotype in a subject comprising: obtaining a biological sample from the subject; detecting the presence of one or more single nucleotide polymorphism (SNP) specific oligonucleotides in serpin family A member 1 (SERPINA1) protein in DNA from the biological sample comprising: performing a first polymerase chain reaction (PCR) reaction comprising specific SERPINA1 primers producing a first PCR product; performing a single SNP-specific primer extension PCR reaction comprising specific SERPINA1 extension primers with the first PCR product producing an extended PCR product; and performing matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on the extended PCR product to detect the SNP-specific oligonucleotide; analyzing the genotype of the detected one or more SNPs; and determining the subject has an abnormal AATD genotype if the one or more SNPs is detected in the sample and is determined not to comprise a normal genotype for AATD.

**[0005]** In one aspect, the subject has a disease or condition selected from the group consisting of emphysema, chronic obstructive pulmonary disease (COPD), asthma, and necrotizing panniculitis.

**[0006]** In still another aspect, the subject is an AATD asymptomatic subject with persistent obstruction on pulmonary function testing having risk factors selected from the group consisting of cigarette smoking and occupational exposure.

**[0007]** In another aspect, the subject is an AATD asymptomatic subject having a family history of AATD.

**[0008]** In yet another aspect, the subject is a newborn.

**[0009]** Another embodiment relates to a kit for detecting an abnormal alpha-1 antitrypsin deficiency (AATD) genotype comprising: (a) at least one primer set capable of detecting SERPINA1 in a biological sample; (b) at least one extension primer set capable of detecting at least one single SNP in SERPINA1 in a biological sample; and an instruction for using the primer set of (a) and the extension primer set of (b), wherein the instruction provides guidance to use the primer set and the extension primer set in performing PCR in detecting SNP-specific oligonucleotides in a biological sample.

**[0010]** In any of the embodiments disclosed herein, the biological sample is selected from the group consisting of whole blood, nasal mucosa and buccal mucosa.

**[0011]** In any of the embodiments disclosed herein, the SNPs that are detected comprise rs6647, rs764325655, rs28929470, rs28931570, rs121912713, rs28931569, rs121912714, rs775982338, rs17580, rs28931568, rs28929474, rs11558261, rs199422209, rs199422211, rs28931572, rs751235320, rs55819880, rs267606950, rs28929473, rs1057516212 or combinations thereof.

### DETAILED DESCRIPTION

**[0012]** This invention generally relates to methods for detecting abnormal AATD genotypes.

**[0013]** The assay disclosed herein is based on PCR followed by a single strand primer-specific extension PCR reaction after shrimp alkaline phosphatase (SAP) cleanup of the first PCR product to generate single nucleotide polymorphisms (SNP) specific oligonucleotides. The detection of these oligo products is performed on matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The PCR and extension primers were designed to target specifically the SNPs listed in Table 1, including common S, Z, less common F and I and other uncommon SNPs. A total of twenty SNPs were multiplexed within one reaction. Each SNP was differentiated by extended oligo products with defined molecular weights between 4000 to 8000 Daltons. In Table 1, “PI typing” stands for “protease inhibitor” typing; “dbSNP ID” stands for “database SNP identification” at the National Center for Biotechnology Information (NCBI); the nucleotide change column provides the NM accession number which links to the mRNA record at the NCBI database and indicates the nucleotide change; the “amino acid change” column provides the specific amino acid change and location (i.e. amino acid position number) of the amino acid change within the sequence. As provided for herein the protein sequence for alpha-1-antitrypsin having NCBI reference number NP\_000286.3 is SEQ ID NO:1. The nucleotide sequence for NCBI reference number NM\_000295.5 is SEQ ID NO:2 and

the nucleotide sequence for NCBI reference number NM\_001127701.2 is SEQ ID NO:3.

and the second allele is “QOludwigshafen”; where in one allele is “QOwest” and the second allele is “QOwest”; where

TABLE 1

SNPs detected by assay			
PI typing	dbSNP ID	Nucleotide change	Amino Acid change
M	rs6647	NM_001127701.1 c.710T > C	p.Val237Ala
QOclayton	rs764325655	NM_000295.5 c.1158dup	p.Glu387fsTer14
F	rs28929470	NM_001127701.1 c.739C > T	p.Arg247Cys
I	rs28931570	NM_001127701.1 c.187C > T	p.Arg63Cys
Pittsburg	rs121912713	NM_001127701.1 c.1145T > G	p.Met382Arg
Mprocida	rs28931569	NM_001127701.1 c.194T > C	p.Leu65Pro
Mplowell	rs121912714	NM_001127701.1 c.839A > T	p.Asp280Val
Mmalton	rs775982338	NM_000295.5 c.227_229delTCT	p.Phe76del
S	rs17580	NM_001127701.1 c.863A > T	p.Glu288Val
Mmineral Springs	rs28931568	NM_001127701.1 c.272G > A	p.Gly91Glu
Z	rs28929474	NM_001127701.1 c.1096G > A	p.Glu366Lys
QOnewport	rs11558261	NM_001127701.1 c.415G > A	p.Gly139Ser
Qheerlen	rs199422209	NM_001127701.1 c.1178C > T	p.Pro393Leu
QObellingham	rs199422211	NM_000295.5 c.721A > T	p.Lys241Ter
QOludwigshafen	rs28931572	NM_001127701.1 c.347T > A	p.Ile116Asn
QOwest	rs751235320	NM_000295.5 c.646 + 1G > T	Protein absence
Siiyama	rs55819880	NM_001127701.1 c.230C > T	p.Ser77Phe
QOgranite Falls	rs267606950	NM_000295.5 c.552del	p.Asp183_Tyr184delinsTer
QOmatawa	rs28929473	NM_001127701.1 c.1131A > T	p.Leu377Phe
QOcasablanca	rs1057516212	NM_000295.5 c.288_291del	p.His97fs

**[0014]** The present inventors have made the surprising discovery that the multiplex genotyping assay disclosed herein accurately detected 20 SNPs (Table 1) and was more accurate than IEF. With the ability to run 96 or 384 samples per run, this assay can be used for a broad AATD screening and diagnosis, especially in people with symptoms of hepatic or lung disorders at any age when there is no obvious cause. The diagnosis can thus also lead to treatment of the subject.

**[0015]** As disclosed herein, a subject having a normal AATD genotype means that only the M allele (or only the M SNIP and none of the other SNPs in Table 1) is detected in a single sample by the assays disclosed herein. A subject having a normal AATD genotype does not have alpha-1 antitrypsin deficiency (AATD) and is a subject with normal alpha-1 antitrypsin proteins (referred to as Pi\*MM). The SNIP in Table 1 for M is rs6647.

**[0016]** As disclosed herein, a subject having an abnormal AATD genotype means that the genotype detected or determined by the assay disclosed herein contains at least one SNP from table 1 that is not M. Examples of abnormal AATD genotypes include but are not limited to: where “M” is one allele and the second allele is any other non-M allele in Table 1; where “F” is one allele and the second allele is “F” or any other allele in Table 1; where “S” is one allele and the second allele is “S” or any other allele in Table 1; where “Z” is one allele and the second allele is “Z” or any other allele in Table 1; where “QOclayton” is one allele and the second allele is “QOclayton”; where one allele is “Mprocida” and the second allele is “Mprocida”; where one allele is “Mplowell” and the second allele is “Mplowell”; where one allele is “Mmalton” and the second allele is “Mmalton”; where one allele is “Mmineral Springs” and the second allele is “Mmineral Springs”; where one allele is “QOnewport” and the second allele is “QOnewport”; where one allele is “Qheerlen” and the second allele is “Qheerlen”; where one allele is “QObellingham” and the second allele is “QObellingham”; where one allele is “QOludwigshafen”

one allele is “Siiyama” and the second allele is “Siiyama”; where one allele is “QOgranite Falls” and the second allele is “QOgranite Falls”; where in one allele is “QOmatawa” and the second allele is “QOmatawa”; and where one allele is “QOcasablanca” and the second allele is “QOcasablanca”.

**[0017]** One embodiment is to determine and/or detect an abnormal alpha-1 antitrypsin deficiency (AATD) genotype in a subject. This assay includes obtaining a biological sample from the subject; detecting the presence of one or more single nucleotide polymorphism (SNP) specific oligonucleotides in serpin family A member 1 (SERPINA1) protein in DNA from the sample. This step comprises performing a first PCR reaction comprising specific SERPINA1 primers producing a first PCR product; then performing a single SNP-specific primer extension PCR reaction comprising specific SERPINA1 extension primers with the first PCR product producing an extended PCR product; and performing matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on the extended PCR product to detect the SNP-specific oligonucleotides. analyzing the genotype of the detected one or more SNPs; determining the subject has an abnormal AATD genotype if the one or more SNPs is detected in the sample and is determined not to comprise a normal genotype for AATD.

**[0018]** Another embodiment relates to a method to genotype AATD in a subject comprising: obtaining a biological sample from the subject; detecting and genotyping SNP specific oligonucleotides in DNA from the biological sample comprising: performing a first PCR reaction comprising SERPINA1 primers producing a first PCR product; performing a single SNP-specific primer extension PCR reaction comprising specific SERPINA1 extension primers with the first PCR product producing an extended PCR product; and performing MALDI-TOF MS on the extended PCR product to detect and genotype the SNP-specific oligonucleotides.

**[0019]** The subject can have a disease or condition selected from the group consisting of emphysema, chronic

obstructive pulmonary disease (COPD), asthma, and necrotizing panniculitis. Further, the subject can be an AATD asymptomatic subject with persistent obstruction on pulmonary function testing having risk factors selected from the group consisting of cigarette smoking and occupational exposure. Further, in one aspect, the subject is an AATD asymptomatic subject having a family history of AATD. In one aspect, the subject is a newborn. The subject may be viewed as a patient. The subject can be a human, and the human can be a patient. The terms, "subject," "patient," and "individual" are used interchangeably and refer to either a human or a non-human animal. These terms include mammals such as humans, primates, livestock animals (e.g., bovines, porcines), companion animals (e.g., canines, felines) and rodents (e.g., mice, rabbits and rats).

**[0020]** The sample can be a sample taken from, or provided by, a subject. Samples may include, but are not limited to, body fluids (e.g., whole blood, dry blood, isolated cells from blood, plasma, serum, cerebrospinal fluid, GI tract contents, semen, urine, stool/fecal); tissues (e.g., nasal mucosa, buccal mucosa, adrenal, hepatic, renal, pancreatic, pituitary, thyroid, immune, ovarian, testicular, prostate, endometrial, ocular, mammary, adipose, epithelial, endothelial, neural, muscle, pulmonary, epidermis, and osseous) as well as samples obtained by e.g. a swab, rinse or scrape of a tissue, body cavity or biopsy.

**[0021]** The term "sample" also encompasses swabs of the abovementioned samples. In the clinical setting, swabs are primarily used to diagnose clinical diseases. The term "sample" encompasses functional equivalents of swabs, regardless of the functional equivalents' sensitivity or reproducibility relative to swabs. In addition to swabs, tissues, cotton balls/pads, wipes, are also encompassed by the term "sample".

**[0022]** The method relates to obtaining DNA from a sample, amplifying a desired region of the DNA, detecting signature characteristics of the amplified region, and identifying the specific SNPs in the sample based on those signature characteristics. First, a sample is obtained from any of a variety of sources, including but not limited to whole blood, dried blood or buccal swabs from tissue. DNA is typically then extracted from the sample, however, the sample can also be used without having first extracted the DNA. One or more regions suspected of being present in the sample is then amplified using primers that anneal to sequences that are specific for the SNPs in Table 1. The amplified regions are then used in PCR based extension reactions. The extension reactions involve using a primer, wherein the primer can be specific to one or more SNPs, to produce an extension reaction product from the amplified region. The extension reaction product will have signature characteristics that can be used to identify and distinguish types of SNPs. The extension product is then analyzed to detect the specific-SNP-specific oligonucleotides in the sample. In some embodiments, the extension reaction product is analyzed to determine its molecular weight and/or mass to charge ratio.

**[0023]** DNA isolation from blood, plasma, or serum can be performed using any method known to one skilled in the art. One such method is disclosed in Chiu, R. W. K. et al. Clin Chem 47:1607-1613. (2001) incorporated herein by reference in its entirety. Other suitable methods include, for example TRI REAGENT® BD (Molecular Research Center, Inc., Cincinnati, Ohio), which is a reagent for isolation of

DNA from, for example, plasma. TRI REAGENT BD and the single-step method are described, for example, in the U.S. Pat. Nos. 4,843,155 and 5,346,994.

**[0024]** Protein levels in the biological sample can be determined by nephelometry.

**[0025]** Polymerase chain reaction, or PCR, is a technique described in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989) and Current Protocols in Molecular Biology, Supplement 1, John Wiley & Sons (1987-1997). Many variations of PCR have been developed, for instance, real time PCR (also known as quantitative PCR or qPCR), hot-start PCR, competitive PCR, and others, and these may all be employed where appropriate.

**[0026]** In some embodiments, using a PCR based amplification, the oligonucleotide primers of the invention are contacted with a reaction mixture containing a target sequence and free nucleotides in a suitable buffer. Thermal cycling of the resulting mixture in the presence of a DNA polymerase results in amplification of the sequence between the primers.

**[0027]** Due to the specificity of the base extension reaction, detection of the SNPs disclosed herein can be accurately reproduced for analysis. This includes differences ranging from single nucleotide variations to small and large deletions, insertions, substitutions, and other types of nucleic acid variations present in even a small percentage of the pool of nucleic acids present in a sample.

**[0028]** The base extension reaction according to the present invention can be performed using any appropriate base extension method. In general, a nucleic acid extension primer is designed to anneal to the target nucleic acid. One or more extension primers of the invention are contacted with a reaction mixture suspected of containing the target sequence, in the presence of free nucleotides, a polymerase enzyme, and any other desired components in a suitable buffer. The extension reaction is then carried out using parameters, such as temperature and duration, that allow synthesis, primed by the extension primer, of an extension reaction product from the corresponding template target sequence.

**[0029]** As with PCR, the parameters used for extension reactions can be adjusted based on the particular components of the reaction, for example for the type of polymerase and the extension primers. However, unlike PCR, extension reactions do not involve thermal cycling to amplify, as opposed to producing a single copy of, a sequence. That is, extension reactions involve only one cycle and produce a single copy of the desired sequence.

**[0030]** The oligonucleotide primers of the present invention can comprise up to 100 nucleotides, preferably up to 80, 60, 50, 40, 30, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, or 6 nucleotides. The oligonucleotide primers may comprise at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 60, or at least 80 nucleotides. The nucleotides of the oligonucleotide can be any type of nucleotide so long as hybridization specificity or efficiency and amplification efficiency is not detrimentally affected. The oligonucleotide may therefore be a deoxyribonucleotide, a ribonucleotide, modifications thereof (e.g. PNA, morpholino-, LNA) and mixtures thereof DNA oligonucleotides and LNA modified DNA oligonucleotides are preferred.

[0031] In some embodiments, the extension reaction products are analyzed by mass spectrometry. The type of mass spectrometry can be any suitable type, including matrix assisted laser desorption ionization (MALDI), electrospray (ES), ion cyclotron resonance (ICR) and Fourier Transform.

[0032] With MALDI mass spectrometry, various mass analyzers can be used, e.g., magnetic sector/magnetic deflection instruments in single or triple quadrupole mode (MS/MS), Fourier transform and time-of-flight (TOF) configurations as is known in the art of mass spectrometry. For the desorption/ionization process, numerous matrix/laser combinations can be used. Ion-trap and reflectron configurations can also be employed. In one embodiment of the present invention, MALDI-TOF is employed to analyze the extension reaction products.

[0033] The methods of the invention are amenable to multiplexing, or the testing of a sample simultaneously. Consistent with the amplification and extension reactions described above, multiple different primers can be used in a single reaction step. That is, more than one primer pair or more than two primers can be used in the amplification reaction, and more than one primer can be used in the extension reaction.

[0034] The present invention also provides kits that include at least one component to assist one in detecting abnormal AATD genotypes. By way of non-limiting example, kits may include at least one SNP detection reagent, such as a primer set (i.e., forward primers and reverse primers that target against amplification near the SNP regions of the SERPINA1). For example, kits are provided for identifying abnormal AATD genotypes. Example kits may include at least one SERPINA primer set containing a forward primer and a reverse primer that are capable of being used to detect a first PCR product. Such a kit further includes at least one extension primer set to produce an extended PCR product and contains a forward primer and a reverse primer that are capable of being used to detect at least one SNP in Table 1. The instructions may include instructions for using the primers in performing PCR in detecting in detecting abnormal AATD genotypes in a biological sample.

[0035] The instructions present in such a kit may instruct the user for example, on how to use the components of the kit to perform the various methods provided herein. In particular, kits may include instructions for detecting SNPs using primer sets.

[0036] Articles of manufacture and kits provided herein may additionally include reagents for carrying out the methods disclosed herein (e.g., buffers, Taq polymerase enzymes, co-factors, and agents to prevent contamination). Such reagents may be specific for one of the commercially available instruments described herein.

[0037] The term “pharmaceutically acceptable carrier” refers to a non-toxic carrier that may be administered to a patient, together with compositions of this invention, and which does not destroy the pharmacological activity of the active agents within the composition. The term “excipient” refers to an additive in a formulation or composition that is not a pharmaceutically active ingredient.

[0038] The term “pharmaceutically effective amount” refers to an amount effective to treat a patient, e.g., effecting a beneficial and/or desirable alteration in the general health of a patient suffering from a disease (including but not limited to cancer or viral or other infection). A “pharmaceu-

tically effective amount” also refers to the amount required to improve the clinical symptoms of a patient.

[0039] Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer (or components) or group of integers (or components), but not the exclusion of any other integer (or components) or group of integers (or components).

[0040] The singular forms “a,” “an,” and “the” include the plurals unless the context clearly dictates otherwise.

[0041] The term “including” is used to mean “including but not limited to.” “Including” and “including but not limited to” are used interchangeably.

[0042] The term “mammal” refers to organisms from the taxonomy class “mammalian,” including but not limited to humans, other primates such as chimpanzees, apes, orangutans and monkeys, rats, mice, cats, dogs, cows, horses, etc.

[0043] Unless otherwise specified, it is to be understood that each embodiment of the invention may be used alone or in combination with any one or more other embodiments of the invention.

[0044] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds, compositions, and methods of use thereof described herein. Such equivalents are considered to be within the scope of the invention.

[0045] The contents of all references, patents and published patent applications cited throughout this Application, as well as their associated figures are hereby incorporated by reference in their entirety. In case of conflict, the present specification, including its specific definitions, will control.

[0046] The following experimental results are provided for purposes of illustration and are not intended to limit the scope of the invention.

## EXAMPLES

### Example 1. Cohort Study with Biobank Samples

[0047] Methods: Blood or buffy coat samples (177), archived in National Jewish Health Biobank with AATD indication, and 176 presumed normal MM (i.e., having MM genotype-see Table 1; PI\*MM or MM) samples were tested. PCR was followed by single SNP-specific primer extension, then SNP-specific extended oligonucleotides were detected by Matrix-Assisted Laser desorption/ionization time-of-flight (MALDI-TOF). Extension primers were designed to include phenotypes:

[0048] PI\*M, PI\*S, PI\*Z, PI\*F, PI\*I, PI\*Pittsburgh, PI\*Mheerlen, PI\*Mmalton, PI\*Mmineral spring, PI\*Mprocida, PI\*Lowell, PI\*QOclayton, PI\*QOBellingham, PI\*QOgranite falls, PI\*QOLudwigshafen, PI\*QOmatawa, PI\*QOwest, PI\*Siiyama, PI\*Newport and H97\* (also referred to as PI\*QOcasablanca).

Twenty SNPs were multiplexed within one reaction. Sanger sequencing was performed to confirm each genotype and to confirm samples with discordant results between phenotyping (IEF) and genotyping by the assay.

[0049] Results: The assay detected all 20 SNPs (see Table 1) to a minimum of 0.5 ng/μl DNA with 100% precision. Sample types include whole blood, dry blood spot and buccal swabs. 89.5% (77/86) agreement was achieved between this assay and historical IEF for samples with known phenotypes. Sanger sequencing confirmed that DNA sequences from 9 discordant samples were in agreement with results from this assay. (Table 2)

TABLE 2

Genotyping results from known phenotyped samples and discordant samples were tested by Sanger sequencing.									
Genotype	MS	MZ	ZZ	MI	SZ	MF	FZ	MM	Total
Sample #	31	39	5	1	5	1	1	3	86
Sample %	36.0%	45.3%	5.8%	1.2%	5.8%	1.2%	1.2%	3.5%	
SAMPLE ID									
Assays	BIO412	BIO574	BIO277	BIO914	BIO729	BIO330	BIO840	BIO520	BIO972
PI from Biobank (IEF)	MS	ZZ	SS	MZ	SS	M	MS	SS	MZ
Genotype, this assay	MM	MZ	MS	MM	MS	MS	SZ	MS	ZZ
Sanger sequencing	MM	MZ	MS	MM, rs70993	MS	MS	SZ	MS	ZZ
*level (Biobank), mg/dL	152	79/97/89	143	75	102	121	75	100	136

\*Normal MM level: 104-276 mg/dL

**[0050]** SNPs were determined for 95.6% (87) of 91 samples from the biobank with unknown phenotype but with AATD indication. Four samples with uncommon genotypes (MMplowell, Mmalton, ZI and MI) and the four samples with MM were confirmed by Sanger sequencing. (Table 3).

TABLE 3

Genotyping results from samples with unknown phenotypes and rare and normal genotypes were confirmed by Sanger sequencing.											
Genotype	MS	MZ	ZZ	MI	SZ	MF	ZI	MMplowell	MmaltonZ	MM	Total
Sample #	22	34	16	1	8	3	1	1	1	4	91
Sample %	24.2	37.4	17.6	1.1	8.8	3.3	1.1	1.1	1.1	4.4	
SAMPLE ID											
(BIO#)	BIO59	BIO133	BIO141	BIO93	BIO91	BIO101	BIO102	BIO124			
Genotype, this assay	MI	ZI	MMplowell	MmaltonZ	MM	MM	MM	MM			
Sanger sequencing	MI	ZI	MMplowell	MmaltonZ	MM	MM	MM	MM			
*level (Biobank), mg/dL	138	74/97	85	<30	139/172	N/A	128	209			

\*Normal MM level: 104-276 mg/dL

**[0051]** From a total cohort of 177 biobank samples, 96.600 (171) were genotyped as abnormal SNPs and confirmed by Sanger sequencing with representation for each genotype. For 176 presumed normal MMN samples, 91% (17) were genotyped as MS, MW, MF, FF or MI (Table 4). All others were genotyped as normal MM. F allele accounts for 1.1% of the group and one FF genotype was identified by this assay and confirmed by Sanger sequencing.

TABLE 4

Summary of presumed normal MM samples.							
TOTAL	FF	MF	MI	MS	MZ	MM	Total Abnormal
176	1	2	1	9	3	160	16
100%	0.6%	1.1%	0.6%	5.1%	1.7%	90.9%	9.1%

[0052] While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following exemplary claims.

---

SEQUENCE LISTING

Sequence total quantity: 3

SEQ ID NO: 1                   moltype = AA   length = 418  
 FEATURE                    Location/Qualifiers  
 source                     1..418  
                           mol\_type = protein  
                           organism = Homo sapiens

SEQUENCE: 1

MPSSVSWGIL	LLAGLCCLVP	VSLAEDPQGD	AAQKTDTS	SHH	DQDHPTFN	KI	TPNLAEFA	FS	60
LYRQLAHQSN	STNIFFSPVS	IATAFAMLSL	GTKADTHDEI	LEGLNFNLT	E	IPEAQIHEGF			120
QELLRTLNP	DSQQLTGN	GLFLSEGLKL	VDKFL	EDVKK	LYHSEAF	TVN	FGDTEEAKKQ		180
INDYVEKGTQ	GKIVDLVKEL	DRDTVFALVN	YIFFK	GKWER	PFEVKDTEEE	DFHVDQVTTV			240
KVPMMKRLGM	FNIQHCKKLS	SWVLLMKYLG	NATAIFFLPD	EGKLQHLENE	LTHDIITKFL				300
ENEDRRSASL	HLPKLSITGT	YDLKSVLGQL	GITKVFSNGA	DLSGVTEEAP	LKLSKAVHKA				360
VLTIDEKGTE	AAGAMFLEAI	PMSIPPEVKF	NKPFVFLMIE	QNTKSPLFMG	KVVNP	TQK			418

SEQ ID NO: 2                   moltype = DNA   length = 3006  
 FEATURE                    Location/Qualifiers  
 source                     1..3006  
                           mol\_type = genomic DNA  
                           organism = Homo sapiens

SEQUENCE: 2

ctcctcagct	tcaggcacca	ccactgacct	gggacagtga	atcgacaatg	ccgtctctctg	60
tctcgtgggg	catcctcctg	ctggcaggcc	tgtgctgect	ggtccctgtc	tccctggctg	120
aggatcccca	gggagatgct	gcccagaaga	cagatacatc	ccaccatgat	caggatcacc	180
caaccttcaa	caagatcacc	cccaacctgg	ctgagttcgc	cttcagccta	taccgccagc	240
tggcacacca	gtccaacagc	accaatatct	tcttctcccc	agtgagcatc	gtacagcct	300
tgcgaatgct	ctccctgggg	accaaggctg	acactcacga	tgaatcctg	gagggcctga	360
atttcaacct	cacggagatt	cgggaggtc	agatccatga	aggcttccag	gaactcctcc	420
gtaccctcaa	ccagccagac	agccagctcc	agctgaccac	cggcaatggc	ctgttcctca	480
gcgagggcct	gaagctagt	gataagtttt	tggaggatgt	taaaaagtgt	taccactcag	540
aagccttcac	tgtcaacttc	ggggacaccg	aagaggccaa	gaacacagatc	aacgattacg	600
tggagaaggg	tactcaaggg	aaaattgtgg	atttgggtcaa	ggagcttgac	agagacacag	660
tttttgctct	ggggaattac	atcttcttta	aaggcaaatg	ggagagaccc	tttgaagtca	720
aggacaccga	ggaagaggac	ttccacgtgg	accaggtgac	caccgtgaag	gtgcctatga	780
tgaagcggtt	aggcatgttt	aacatccagc	actgtaagaa	gctgtccagc	tgggtgctgc	840
tgatgaata	ctcggggaat	gccaccgcca	tcttcttctc	gcctgatgag	gggaaactac	900
agcacctgga	aaatgaactc	accacagata	tcatcaccaa	gttcctggaa	aatgaagaca	960
gaaggtctgc	cagcttacat	ttaccctaac	tgtccattac	tggaaacctat	gatctgaaga	1020
gcgtcctggg	tcaactgggc	atcactaagg	tcttcagcaa	tggggctgac	ctctccgggg	1080
tcacagagga	ggcaccctcg	aagctctcca	aggccgtgca	taaggctgtg	ctgaccatcg	1140
acgagaaagg	gactgaagct	gctggggcca	tgtttttaga	ggccataccc	atgtctatcc	1200
ccccgaggt	caagttcaac	aaacctttg	tcttcttaat	gattgaacaa	aataccaagt	1260
ctcccctctt	catgggaaaa	gtggtgaatc	ccaccctaaa	ataactgcct	ctcgtcctc	1320
aaccctctcc	ctccatccct	ggccccctcc	ctggatgaca	ttaaagaagg	gttgagctgg	1380
tccctgcctg	catgtgactg	taaatccctc	ccatgttttc	tctgagtcct	cctttgcctg	1440
ctgaggtctg	atgtgggctc	caggtaacag	tgtgtctctc	gggccccctg	aactgtgttc	1500
atggagcatc	tggctgggta	ggcacatgct	gggcttgaat	ccagggggga	ctgaatcctc	1560
agcttacgga	cctgggcccc	tctgtttctg	gagggctcca	gtcttctctg	tctgtctctg	1620
gagtcctcaa	gaaggaatca	caggggagga	accagatacc	agccatgacc	ccaggctcca	1680
ccaagcatct	tcatgtcccc	ctgctcatcc	cccactcccc	cccaccacga	gttgctcatc	1740
ctgccagggc	tggctgtgcc	caccctcaag	ctgccctcct	ggggggcccc	gaactcctg	1800
atcgtgccgt	ggccaggttt	tgtggcatct	gcagcaaac	aagagagagg	acaatgtcct	1860
cctcttgacc	cgtgtgcacc	taaccagact	cgggccccgc	acctctcagg	cacttctgga	1920
aaatgactga	ggcagattct	tctgaagcc	cattctccat	ggggcaacaa	ggacacctat	1980
tctgtccttg	tccttccatc	gctgccccag	aaagcctcac	atatctccgt	ttagaatcag	2040
gtcccttctc	cccagatgaa	gaggagggtc	tctgctttgt	tttctctatc	tcctcctcag	2100
acttgaccag	gccagccagg	cccagaaga	ccattaccct	atatcccttc	tcctccctag	2160
tcacatggcc	ataggcctgc	tgatggctca	ggaaggccat	tgcaaggact	cctcagctat	2220
gggagaggaa	gcacatcacc	cattgacccc	cgaacccctc	cccttctctc	ctctgagctc	2280
cgaactgggg	cacatgcagc	ctgacttctt	tgtgctctgt	gctgtccctg	cagtcttcag	2340
agggccaccg	cagctccagt	gccacggcag	gaggctgttc	ctgaatagcc	cctgtggtaa	2400
gggcaggag	agtccttcca	tcttccaaag	ccctgctaaa	ggacacagca	gccagggaagt	2460
cccctgggcc	cctagctgaa	ggacagcctg	ctccctccgt	ctctaccagg	aatggccttg	2520
tcctatggaa	ggcactgcct	catcccaaac	taactctagga	atcactgtct	aacctctcac	2580
tgtcatgaat	gtgtacttaa	aggatgaggt	tgagtcatac	caaatagtga	tttcgatagt	2640

-continued

tcaaaatggt	gaaattagca	attctacatg	attcagtcga	atcaatggat	accgactgtt	2700
tcccacacaa	gtctctgtgt	ctcttaagct	tactcactga	cagcctttca	ctctccacaa	2760
atacattaaa	gatatggcca	tcaccaagcc	ccttaggatg	acaccagacc	tgagagtctg	2820
aagacctgga	tccaagttct	gacttttccc	cctgacagct	gtgtgacctt	cgtgaagtgc	2880
ccaaacctct	ctgagcccca	gtcattgcta	gtaagacctg	cctttgagtt	ggtatgatgt	2940
tcaagttaga	taacaaaatg	tttataccca	ttagaacaga	gaataaatag	aactacattt	3000
cttgca						3006

SEQ ID NO: 3                   moltype = DNA   length = 3475  
 FEATURE                   Location/Qualifiers  
 source                   1..3475  
                           mol\_type = genomic DNA  
                           organism = Homo sapiens

SEQUENCE: 3

agagtcctga	gctgaaccaa	gaaggaggag	ggggtcgggc	ctccgaggaa	ggcctagccg	60
ctgctgctgc	caggaattcc	aggttggagg	ggcggaacc	tcctgccagc	cttcaggcca	120
ctctcctgtg	cctccagaaa	ggagcagagc	ttgaggagag	cttgaggaga	gcaggaaagg	180
tgggacattg	ctgctgctgc	tcactcagtt	ccacagggcg	gcagtaagtc	ttcagcatca	240
ggcatttttg	ggtgactcag	taaatggtag	atcttgctac	cagtggaaac	gccactaagg	300
attctgcagt	gagagcagag	ggccagctaa	gtggtactct	cccagagact	gtctgactca	360
cgccaccccc	tcacacctgg	acacaggagc	ctgtgggttc	tgagccagca	gcctcccccg	420
ttgcccctct	ggatccactg	cttaaatatc	gacgaggaca	gggcccctgc	tcctcagctt	480
caggcaccac	cactgacctg	ggacagtga	tcgacaatgc	cgtctcttgt	ctcgtggggc	540
atcctcctgc	tggcaggcct	gtgctgcctg	gtccctgtct	ccctggctga	ggatccccag	600
ggagatgctg	cccagaagac	agatacatcc	caccatgatc	aggatcacc	aaccttcaac	660
aagatcacc	ccaacctggc	tgagttcgcc	ttcagcctat	accgccagct	ggcacaccag	720
tccaacagca	ccaatatctt	cttctcccca	gtgagcatcg	ctacagcctt	tgcaatgtct	780
tccttgggga	ccaaggctga	cactcacgat	gaaatcctgg	agggcctgaa	tttcaacctc	840
acggagattc	cggaggctca	gatccatgaa	ggcttcacag	aactcctccg	taccctcaac	900
cagccagaca	gccagctcca	gctgaccacc	ggcaatggcc	tgttctctcag	cgaggggcctg	960
aagctagtgg	ataagttttt	ggaggatggt	aaaaagttgt	accactcaga	agccttcaact	1020
gtcaacttcg	gggacaccga	agaggccaag	aaacagatca	acgattacgt	ggagaagggt	1080
actcaaggga	aaattgtgga	ttgggtcaag	gagcttgaca	gagacacagt	ttttgtctctg	1140
gtgaattaca	tctcttttaa	aggcaaatgg	gagagaccct	ttgaagtcaa	ggacaccgag	1200
gaagaggact	tcacgtgga	ccaggtgacc	accgtgaagg	tgcttatgat	gaagcgttta	1260
ggcatgttta	acatccagca	ctgtaagaag	ctgtccagct	gggtgctgct	gatgaaatac	1320
ctgggcaatg	ccaccgccat	cttcttctctg	cctgatgagg	ggaaactaca	gcacctggaa	1380
aatgaactca	cccacgatat	catcaccaag	ttcctggaaa	atgaagacag	aagggtctgcc	1440
agcttacatt	taccctaaact	gtccattact	ggaacctatg	atctgaagag	cgtcctgggt	1500
caactgggca	tcactaaggt	cttcagcaat	ggggctgacc	tctccggggt	cacagaggag	1560
gcaccctga	agctctccaa	ggcgtgcat	aaggctgtgc	tgacctcga	cgagaaggag	1620
actgaagctg	ctggggccat	gttttttagag	gccataccca	tgtctatccc	ccccgaggtc	1680
aagttcaaca	aaccttttgt	ctcttaaatg	attgaacaaa	ataccaagtc	tccctctctc	1740
atgggaaaaa	gggtgaatcc	cacccaaaaa	taactgcctc	tcgctcctca	acccctcccc	1800
tcctccctcg	ggccctctcc	tggatgacat	taaagaaggg	ttgagctggt	ccctgctctgc	1860
atgtgactgt	aaatccctcc	catgttttct	ctgagctctc	ctttgctctg	tgaggctgta	1920
tgtgggtctc	aggtaacagt	gctgtctctg	ggccccctga	actgtgttca	tggagcatct	1980
ggctgggtag	gcacatgctg	ggcttgaatc	caggggggac	tgaatcctca	gcttacggac	2040
ctgggcccac	ctgtttcttg	agggtccag	tcttctctgt	cctgtcttgg	agtccccaa	2100
aagggaatc	aggggaggaa	ccagatacca	gccatgaccc	caggctccac	caagcatctt	2160
catgtccccc	tgcctatccc	ccactcccc	ccaccagag	ttgctcatcc	tgccagggtc	2220
ggctgtgccc	accccaaggc	tgccctctctg	ggggccccag	aactgctga	tcgtgccgtg	2280
gcccagtttt	gtggcatctg	cagcaacaca	agagagagga	caatgtcctc	ctcttgaccc	2340
gctgtcacct	aaccagactc	gggcccctga	cctctcaggc	acttctggaa	aatgactgag	2400
gcagattctt	cctgaagccc	attctccatg	gggcaacaag	gacacctatt	ctgtccttgt	2460
ccttccatcg	ctgcccacaga	aagcctcaca	tatctccgtt	tagaatcagg	tccctctctc	2520
ccagatgaag	aggagggtct	ctgcttttgt	ttctctatct	cctcctcaga	cttgaccagg	2580
cccagcaggc	cccagaagac	cattacccta	tatccctctc	cctccctagt	cacatggcca	2640
taggcctgct	gatggctcag	gaaggccatt	gcaaggactc	ctcagctatg	ggagaggga	2700
cacatcaccc	attgaccccc	gcaacccctc	ccttctctcc	tctgagctcc	gactggggcc	2760
acatgcagcc	tgacttcttt	gtgctgtgtg	ctgtccctgc	agtcttcaga	gggccaccgc	2820
agctccagtg	ccacggcagg	aggctgttcc	tgaatagccc	ctgtggtaag	ggccaggaga	2880
gtccttccat	cctccaaggc	cctgctaaag	gacacagcag	ccagggaagt	ccctggggcc	2940
ctagctgaag	gacagcctgc	tcctccctgc	tctaccagga	atggccttgt	cctatggaag	3000
gcactgcccc	atcccaact	aactcaggaa	tcaactgtcta	accactcact	gtcatgaatg	3060
tgtacttaaa	ggatgaggtt	gagtcatacc	aaatagtgat	ttcgatagtt	caaaatgggtg	3120
aaatagcaaa	ttctacatga	ttcagtcata	tcaatggata	ccgactgttt	cccacacaag	3180
tctcctgttc	tcttaagctt	attcactgac	agcctttcac	tctccacaaa	tacattaaag	3240
atatggccat	caccaagccc	cctaggatga	caccagacct	gagagctctga	agacctggat	3300
ccaagttctg	acttttcccc	ctgacagctg	tgtgaccttc	gtgaagtgcg	caaacctctc	3360
tgagccccag	tcattgctag	taagacctgc	ctttgagttg	gtatgatggt	caagtttagat	3420
aacaaaatgt	ttatacccat	tagaacagag	aataaataga	actacatttc	ttgca	3475



What is claimed:

1. A method to detect an abnormal alpha-1 antitrypsin deficiency (AATD) genotype in a subject comprising:

- a. obtaining a biological sample from the subject;
- b. detecting the presence of one or more single nucleotide polymorphism (SNP) specific oligonucleotides in serpin family A member 1 (SERPINA1) protein in DNA from the biological sample comprising:

- 1. performing a first polymerase chain reaction (PCR) reaction comprising specific SERPINA1 primers producing a first PCR product;
- 2. performing a single SNP-specific primer extension PCR reaction comprising specific SERPINA1 extension primers with the first PCR product producing an extended PCR product; and
- 3. performing matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on the extended PCR product to detect the SNP-specific oligonucleotide;

- c. analyzing the genotype of the detected one or more SNPs;

- d. determining the subject has an abnormal AATD genotype if the one or more SNPs is detected in the sample and is determined not to comprise a normal genotype for AATD.

2. The method of claim 1, wherein the subject has a disease or condition selected from the group consisting of emphysema, chronic obstructive pulmonary disease (COPD), asthma, and necrotizing panniculitis.

3. The method of claim 1, wherein the subject is an asymptomatic subject with persistent obstruction on pulmonary function testing having risk factors selected from the group consisting of cigarette smoking and occupational exposure.

4. The method of claim 1, wherein the subject is an AATD asymptomatic subject having a family history of AATD.

5. The method of claim 1, wherein in the subject is a newborn.

6. The method of claim 1, wherein the biological sample is selected from the group consisting of whole blood, dry blood, isolated cells from blood, nasal mucosa and buccal mucosa.

7. The method of claim 1, wherein the SNPs comprise rs6647, rs764325655, rs28929470, rs28931570, rs121912713, rs28931569, rs121912714, rs775982338, rs17580, rs28931568, rs28929474, rs11558261, rs199422209, rs199422211, rs28931572, rs751235320, rs55819880, rs267606950, rs28929473, rs1057516212 or a combination thereof.

8. A kit for detecting an abnormal alpha-1 antitrypsin deficiency (AATD) genotype comprising:

- a. at least one primer set capable of detecting SERPINA1 in a biological sample;

- b. at least one extension primer set capable of detecting at least one single SNP in SERPINA1 in a biological sample; and

- c. an instruction for using the primer set of (a) and the extension primer set of (b), wherein the instruction provides guidance to use the primer set and the extension primer set in performing PCR in detecting SNP-specific oligonucleotides in a biological sample.

9. The kit of claim 8, wherein the biological sample is selected from the group consisting of whole blood, dry blood, isolated cells from blood, nasal mucosa and buccal mucosa.

10. The kit of claim 8, wherein the SNPs comprise rs6647, rs764325655, rs28929470, rs28931570, rs121912713, rs28931569, rs121912714, rs775982338, rs17580, rs28931568, rs28929474, rs11558261, rs199422209, rs199422211, rs28931572, rs751235320, rs55819880, rs267606950, rs28929473, rs1057516212 or a combination thereof.

\* \* \* \* \*