"METHODS AND COMPOSITIONS FOR ASSESSMENT OF PULMONARY FUNCTION AND DISORDERS"

FIELD OF THE INVENTION

The present invention is concerned with methods for assessment of pulmonary function and/or disorders, and in particular for assessing risk of developing chronic obstructive pulmonary disease (COPD) and emphysema in smokers and non-smokers using analysis of genetic polymorphisms and altered gene expression. The present invention is also concerned with the use of genetic polymorphisms in the assessment of a subject's risk of developing COPD and emphysema.

BACKGROUND OF THE INVENTION

Chronic obstructive pulmonary disease (COPD) is the 4th leading cause of death in developed countries and a major cause for hospital readmission world-wide. It is characterised by insidious inflammation and progressive lung destruction. It becomes clinically evident after exertional breathlessness is noted by affected smokers when 50% or more of lung function has already been irreversibly lost. This loss of lung function is detected clinically by reduced expiratory flow rates (specifically forced expiratory volume in one second or FEV1). Over 95% of COPD is attributed to cigarette smoking yet only 20% or so of smokers develop COPD (susceptible smoker). Studies surprisingly show that smoking dose accounts for only about 16% of the impaired lung function. A number of family studies comparing concordance in siblings (twins and non-twin) consistently show a strong familial tendency and the search for COPD disease-susceptibility (or disease modifying) genes is underway.

Despite advances in the treatment of airways disease, current therapies do not significantly alter the natural history of COPD with progressive loss of lung function causing respiratory failure and death. Although cessation of smoking has been shown to reduce this decline in lung function if this is not achieved within the first 20 years or so of smoking for susceptible smokers, the loss is considerable and symptoms of worsening breathlessness cannot be averted. Smoking cessation studies indicate that techniques to help smokers quit have limited success. Analogous to the discovery of serum cholesterol and its link to coronary artery disease, there is a need to better understand the factors that contribute to COPD so that tests that identify at risk smokers can be developed and that new treatments can be discovered to reduce the adverse effects of smoking.

A number of epidemiology studies have consistently shown that at exposure doses of 20 or more pack years, the distribution in lung function tends toward trimodality with a proportion of smokers maintaining normal lung function (resistant smokers) even after 60+ pack years, a proportion showing modest reductions in lung function who may never develop symptoms and a proportion who show an accelerated loss in lung function who invariably develop COPD. This suggests that amongst smokers 3 populations exist, those resistant to developing COPD, those at modest risk and those at higher risk (termed susceptible smokers).

COPD is a heterogeneous disease encompassing, to varying degrees, emphysema and chronic bronchitis which develop as part of a remodelling process following the inflammatory insult from chronic tobacco smoke exposure and other air pollutants. It is likely that many genes are involved in the development of COPD.

To date, a number of biomarkers useful in the diagnosis and assessment of propensity towards developing various pulmonary disorders have been identified. These include, for example, single nucleotide polymorphisms including the following: A-82G in the promoter of the gene encoding human macrophage elastase (MMP12); T→C within codon 10 of the gene encoding transforming growth factor beta (TGFβ); C+760G of the gene encoding superoxide dismutase 3 (SOD3); T-1296C within the promoter of the gene encoding tissue inhibitor of metalloproteinase 3 (TIMP3); and polymorphisms in linkage disequilibrium (LD) with these polymorphisms, as disclosed in PCT International Application PCT/NZ02/00106 (published as WO 02/099134 and incorporated herein in its entirety).

It would be desirable and advantageous to have additional biomarkers which could be used to assess a subject's risk of developing pulmonary disorders such as chronic obstructive pulmonary disease (COPD) and emphysema, or a risk of developing COPD/emphysema-related impaired lung function, particularly if the subject is a smoker.

It is primarily to such biomarkers and their use in methods to assess risk of developing such disorders that the present invention is directed.

SUMMARY OF THE INVENTION

The present invention is primarily based on the finding that certain polymorphisms are found more often in subjects with COPD, emphysema, or both COPD and emphysema than in control subjects. Analysis of these polymorphisms

reveals an association between genotypes and the subject's risk of developing COPD, emphysema, or both COPD and emphysema.

Thus, according to one aspect there is provided a method of determining a subject's risk of developing one or more obstructive lung diseases comprising analysing a sample from said subject for the presence or absence of one or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin; or

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only;

wherein the presence or absence of one or more of said polymorphisms is indicative of the subject's risk of developing one or more obstructive lung diseases selected from the group consisting of chronic obstructive pulmonary disease (COPD), emphysema, or both COPD, emphysema, or both COPD and emphysema.

The one or more polymorphisms can be detected directly or by detection of one or more polymorphisms which are in linkage disequilibrium with said one or more polymorphisms.

Linkage disequilibrium (LD) is a phenomenon in genetics whereby two or more mutations or polymorphisms are in such close genetic proximity that they are co-inherited. This means that in genotyping, detection of one polymorphism as present infers the presence of the other. (Reich DE et al; Linkage disequilibrium in the human genome, Nature 2001, 411:199-204.)

The method can additionally comprise analysing a sample from said subject for the presence of one or more further polymorphisms selected from the group consisting of:

16Arg/Gly in the gene encoding β2 Adrenergic Receptor (ADBR);

130 Arg/Gln (G/A) in the gene encoding Interleukin13 (IL13);

298 Asp/Glu (T/G) in the gene encoding Nitric oxide Synthase 3 (NOS3);

Ile 105 Val (A/G) in the gene encoding Glutathione S Transferase P (GST-P);

Glu 416 Asp (T/G) in the gene encoding Vitamin D binding protein (VDBP);

Lys 420 Thr (A/C) in the gene encoding VDBP;

-1055 C/T in the promoter of the gene encoding IL13;

-308 G/A in the promoter of the gene encoding TNFα;

-511 A/G in the promoter of the gene encoding Interleukin 1B (IL1B);

Tyr 113 His T/C in the gene encoding Microsomal epoxide hydrolase (MEH);

His139 Arg G/A in the gene encoding MEH;

Gln 27 Glu C/G in the gene encoding ADBR;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1) with reference to the 2G allel only;

-1562 C/T in the promoter of the gene encoding Metalloproteinase 9 (MMP9);

M1 (GSTM1) null in the gene encoding Glutathione S Transferase 1 (GST-1);

1237 G/A in the 3' region of the gene encoding α1-antitrypsin;

-82 A/G in the promoter of the gene encoding MMP12;

T→C within codon 10 of the gene encoding TGFβ;

760 C/G in the gene encoding SOD3;

-1296 T/C within the promoter of the gene encoding TIMP3; or

the S mutation in the gene encoding α1-antitrypsin.

Again, detection of the one or more further polymorphisms may be carried out directly or by detection of polymorphisms in linkage disequilibrium with the one or more further polymorphisms.

The presence of one or more polymorphisms selected from the group consisting of:

the -765 CC or CG genotype in the promoter of the gene encoding COX2;

the 130 Arg/Gln AA genotype in the gene encoding IL13;

the 298 Asp/Glu TT genotype in the gene encoding NOS3;

the Lys 420 Thr AA or AC genotype in the gene encoding VDBP;

the Glu 416 Asp TT or TG genotype in the gene encoding VDBP;

the Ile 105 Val AA genotype in the gene encoding GSTP-1;

the MS genotype in the gene encoding α1-antitrypsin;

the +489 GG geneotype in the gene encoding TNFα;

the -308 GG geneotype in the gene encoding TNFα;

the C89Y AA or AG geneotype in the gene encodoing SMAD3;

the 161 GG genotype in the gene encodoing MBL2;

the -1903 AA genotype in the gene encoding CMA1;

the Arg 197 Gln AA genotype in the gene encoding NAT2;

the His 139 Arg GG genotype in the gene encoding MEH;

the -366 AA or AG genotype in the gene encoding ALOX5;

the HOM T2437C TT genotype in the gene encoding HSP 70;

the exon 1 +49 CT or TT genotype in the gene encoding Elafin;

the Gln 27 Glu GG genotype in the gene encoding ADBR; or

the -1607 1G1G or 1G2G genotype in the promoter of the gene encoding MMP1;

may be indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

The presence of one or more polymorphisms selected from the group consisting of:

the 105 AA genotype in the gene encoding IL18;

the -133 CC genotype in the promoter of the gene encoding IL18;

the -675 5G5G genotype in the promoter of the gene encoding PAI-1;

the -1055 TT genotype in the promoter of the gene encoding IL13;

the 874 TT genotype in the gene encoding IFN–γ;

the +489 AA or AG genotype in the gene encoding TNFα;

the -308 AA or AG genotype in the gene encoding TNFα;

the C89Y GG genotype in the gene encoding SMAD3;

the E469K GG genotype in the gene encoding ICAM1;

the Gly 881 Arg GC or CC genotype in the gene encoding NOD2;

the -511 GG genotype in the gene encoding IL1B;

the Tyr 113 His TT genotype in the gene encoding MEH;

the -366 GG genotype in the gene encoding ALOX5;

the HOM T2437C CC or CT genotype in the gene encoding HSP 70;

the +13924 AA genotype in the gene encoding CLCA1; or

the -159 CC genotype in the gene encoding CD-14;

may be indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

The methods of the invention are particularly useful in smokers (both current and former).

It will be appreciated that the methods of the invention identify two categories of polymorphisms – namely those associated with a reduced risk of developing COPD, emphysema, or both COPD and emphysema (which can be termed "protective polymorphisms") and those associated with an increased risk of developing COPD, emphysema, or both COPD and emphysema (which can be termed "susceptibility polymorphisms").

Therefore, the present invention further provides a method of assessing a subject's risk of developing chronic obstructive pulmonary disease (COPD), emphysema, or both COPD and emphysema, said method comprising:

determining the presence or absence of at least one protective polymorphism associated with a reduced risk of developing COPD, emphysema, or both COPD and emphysema; and

in the absence of at least one protective polymorphism, determining the presence or absence of at least one susceptibility polymorphism associated with an increased risk of developing COPD, emphysema, or both COPD and emphysema;

wherein the presence of one or more of said protective polymorphisms is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema, and the absence of at least one protective polymorphism in combination with the presence of at least one susceptibility polymorphism is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

Preferably, said at least one protective polymorphism is selected from the group consisting of:

-765 C in the promoter of the gene encoding COX2;

130 Arg/Gln A in the gene encoding IL13;

298 Asp/Glu T in the gene encoding NOS3;

Lys 420 Thr A in the gene encoding VDBP;

Glu 416 Asp T in the gene encoding VDBP;

Ile 105 Val A in the gene encoding GSTP-1;

the S mutation in the gene encoding α1-antitrypsin;

+489 G in the gene encoding TNFα;

-308 G in the gene encoding TNFα;

C89Y A in the gene encoding SMAD3;

161 G in the gene encoding MBL2;

-1903 A in the gene encoding CMA1;

Arg 197 Gln A in the gene encoding NAT2;

His 139 Arg G in the gene encoding MEH;

-366 A in the gene encoding ALOX5;

HOM 2437 T in the gene encoding HSP 70;

exon 1 +49 T in the gene encodoing Elafin;

Gln 27 Glu G in the gene encoding ADBR; or

-1607 1G in the promoter of the gene encoding MMP1.

In another embodiment, said at least one protective polymorphism is a genotype selected from the group consisting of:

the -765 CC or CG genotype in the promoter of the gene encoding COX2;

the 130 Arg/Gln AA genotype in the gene encoding IL13;

the 298 Asp/Glu TT genotype in the gene encoding NOS3;

the Lys 420 Thr AA or AC genotype in the gene encoding VDBP;

the Glu 416 Asp TT or TG genotype in the gene encoding VDBP;

the Ile 105 Val AA genotype in the gene encoding GSTP-1;

the MS genotype in the gene encoding α1-antitrypsin;

the +489 GG geneotype in the gene encoding TNFα;

the -308 GG geneotype in the gene encoding TNFα;

the C89Y AA or AG geneotype in the gene encodoing SMAD3;

the 161 GG genotype in the gene encodoing MBL2;

the -1903 AA genotype in the gene encoding CMA1;

the Arg 197 Gln AA genotype in the gene encoding NAT2;

the His 139 Arg GG genotype in the gene encoding MEH;

the -366 AA or AG genotype in the gene encoding ALOX5;

the HOM T2437C TT genotype in the gene encoding HSP 70;

the exon 1 +49 CT or TT genotype in the gene encoding Elafin;

the Gln 27 Glu GG genotype in the gene encoding ADBR; or

the -1607 1G1G or 1G2G genotype in the promoter of the gene encoding MMP1.

Optionally, said method comprises the additional step of determining the presence or absence of at least one further protective polymorphism selected from the group consisting of:

the +760GG or +760CG genotype within the gene encoding SOD3;

the -1296TT genotype within the promoter of the gene encoding TIMP3; or

the CC genotype (homozygous P allele) within codon 10 of the gene encoding TGFβ.

The at least one susceptibility polymorphism may be a genotype selected from the group consisting of:

the 105 AA genotype in the gene encoding IL18;

the -133 CC genotype in the promoter of the gene encoding IL18;

the -675 5G5G genotype in the promoter of the gene encoding PAI-1;

the -1055 TT genotype in the promoter of the gene encoding IL13;

the 874 TT genotype in the gene encoding IFN–γ;

the +489 AA or AG genotype in the gene encoding TNFα;

the -308 AA or AG genotype in the gene encoding TNFα;

the C89Y GG genotype in the gene encoding SMAD3;

the E469K GG genotype in the gene encoding ICAM1;

the Gly 881 Arg GC or CC genotype in the gene encoding NOD2;

the -511 GG genotype in the gene encoding IL1B;

the Tyr 113 His TT genotype in the gene encoding MEH;

the -366 GG genotype in the gene encoding ALOX5;

the HOM T2437C CC or CT genotype in the gene encoding HSP 70;

the +13924 AA genotype in the gene encoding CLCA1; or

the -159 CC genotype in the gene encoding CD-14.

Optionally, said method comprises the step of determining the presence or absence of at least one further susceptibility polymorphism selected from the group consisting of:

the -82AA genotype within the promoter of the gene encoding MMP12;

the -1562CT or -1562TT genotype within the promoter of the gene encoding MMP9;

the 1237AG or 1237AA genotype (Tt or tt allele genotypes) within the 3' region of the gene encoding α 1-antitrypsin; or

the 2G2G genotype within the promoter of the gene encoding MMP1.

In a preferred form of the invention the presence of two or more protective polymorphisms is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

In a further preferred form of the invention the presence of two or more susceptibility polymorphisms is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

In still a further preferred form of the invention the presence of two or more protective polymorphims irrespective of the presence of one or more susceptibility polymorphisms is indicative of reduced risk of developing COPD, emphysema, or both COPD and emphysema.

In another aspect, the invention provides a method of determining a subject's risk of developing COPD, emphysema, or both COPD and emphysema, said method comprising obtaining the result of one or more genetic tests of a sample from said subject, and analysing the result for the presence or absence of one or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only;

or one or more polymorphisms which are in linkage disequilibrium with any one or more of these polymorphisms;

wherein a result indicating the presence or absence of one or more of said polymorphisms is indicative of the subject's risk of developing COPD, emphysema, or both COPD and emphysema.

In a further aspect the invention provides a method of determining a subject's risk of developing chronic obstructive pulmonary disease (COPD), emphysema, or both COPD and emphysema, said method comprising determining the presence or absence of the -765 C allele in the promoter of the gene encoding COX2 and/or the S allele in the gene encoding 1-antitrypsin, wherein the presence of any one or more of said alleles is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

In a further aspect the invention provides a method of determining a subject's risk of developing chronic obstructive pulmonary disease (COPD), emphysema, or both COPD and emphysema, said method comprising determining the presence or absence of the -765 CC or CG genotype in the promoter of the gene encoding COX2 and/or the MS genotype in the gene encoding 1-antitrypsin, wherein the presence of any one or more of said genotypes is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

In one particularly preferred form of the invention there is provided a method of determining a subject's risk of developing chronic obstructive pulmonary disease (COPD), emphysema, or both COPD and emphysema, comprising the analysis of one or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding COX2;

105 C/A in the gene encoding IL18;

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding PAI-1;

874 A/T in the gene encoding IFN-γ;

+489 G/A in the gene encoding TNFα;

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding ICAM1;

Gly 881Arg G/C in the gene encoding NOD2;

161 G/A in the gene encoding MBL2;

-1903 G/A in the gene encoding CMA1;

Arg 197 Gln G/A in the gene encoding NAT2;

-366 G/A in the gene encoding ALOX5;

HOM T2437C in the gene encoding HSP 70;

+13924 T/A in the gene encoding CLCA1;

-159 C/T in the gene encoding CD-14;

exon 1 +49 C/T in the gene encoding Elafin; or

-1607 1G/2G in the promoter of the gene encoding MMP1 (with reference to the 1G allele only)

in combination with one or more polymorphisms selected from the group consisting of:

16Arg/Gly in the gene encoding ADBR;

130 Arg/Gln (G/A) in the gene encoding IL13;

298 Asp/Glu (T/G) in the gene encoding NOS3;

Ile 105 Val (A/G) in the gene encoding GSTP;

Glu 416 Asp (T/G) in the gene encoding VDBP;

Lys 420 Thr (A/C) in the gene encoding VDBP;

-1055 C/T in the promoter of the gene encoding IL13;

the S mutation in the gene encoding α1-antitrypsin;

-308 G/A in the promoter of the gene encoding TNFα;

-511 A/G in the promoter of the gene encoding IL1B;

Tyr 113 His T/C in the gene encoding MEH;

His 139 Arg G/A in the gene encoding MEH; or

Gln 27 Glu C/G in the gene encoding ADBR.

In a further aspect there is provided a method of determining a subject's risk of developing chronic obstructive pulmonary disease (COPD), emphysema, or both COPD and emphysema, comprising the analysis of two or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding COX2;

105 C/A in the gene encoding IL18;

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding PAI-1;

874 A/T in the gene encoding IFN-γ;

16Arg/Gly in the gene encoding ADBR;

130 Arg/Gln (G/A) in the gene encoding IL13;

298 Asp/Glu (T/G) in the gene encoding NOS3;

Ile 105 Val (A/G) in the gene encoding glutathione S transferase P (GST-P);

Glu 416 Asp (T/G) in the gene encoding VDBP;

Lys 420 Thr (A/C) in the gene encoding VDBP;

-1055 C/T in the promoter of the gene encoding IL13;

the S mutation in the gene encoding α1-antitrypsin;

+489 G/A in the gene encoding TNFα;

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding ICAM1;

Gly 881Arg G/C in the gene encoding NOD2;

161 G/A in the gene encoding MBL2;

-1903 G/A in the gene encoding CMA1;

Arg 197 Gln G/A in the gene encoding NAT2;

-366 G/A in the gene encoding ALOX5;

HOM T2437C in the gene encoding HSP 70;

+13924 T/A in the gene encoding CLCA1;

-159 C/T in the gene encoding CD-14;

exon 1 +49 C/T in the gene encoding Elafin;

-308 G/A in the promoter of the gene encoding TNFα;

-511 A/G in the promoter of the gene encoding IL1B;

Tyr 113 His T/C in the gene encoding MEH;

Arg 139 G/A in the gene encoding MEH;

Gln 27 Glu C/G in the gene encoding ADBR; or

-1607 1G/2G in the promoter of the gene encoding MMP1 (with reference to the 1G allele only).

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 298 of the gene encoding NOS3.

The presence of glutamate at said position is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

The presence of asparagine at said position is indicative of reduced risk of developing COPD, emphysema, or both COPD and emphysema.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 420 of the gene encoding vitamin D binding protein.

The presence of threonine at said position is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

The presence of lysine at said position is indicative of reduced risk of developing COPD, emphysema, or both COPD and emphysema.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 89 of the gene encoding SMAD3.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 469 of the gene encoding ICAM1.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 881 of the gene encoding NOD2.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 197 of the gene encoding NAT2.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 113 of the gene encoding MEH.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 139 of the gene encoding MEH.

In various embodiments, any one or more of the above methods comprises the step of analysing the amino acid present at a position mapping to codon 27 of the gene encoding ADBR.

In a preferred form of the invention the methods as described herein are performed in conjunction with an analysis of one or more risk factors, including one or

more epidemiological risk factors, associated with a risk of developing chronic obstructive pulmonary disease (COPD) and/or emphysema. Such epidemiological risk factors include but are not limited to smoking or exposure to tobacco smoke, age, sex, and familial history of COPD, emphysema, or both COPD and emphysema.

In a further aspect, the invention provides for the use of at least one polymorphism in the assessment of a subject's risk of developing COPD, emphysema, or both COPD and emphysema, wherein said at least one polymorphism is selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only; or

one or more polymorphisms in linkage disequilibrium with any one of said polymorphisms.

Optionally, said use may be in conjunction with the use of at least one further polymorphism selected from the group consisting of:

16Arg/Gly in the gene encoding ADBR;

130 Arg/Gln (G/A) in the gene encoding IL13;

298 Asp/Glu (T/G) in the gene encoding NOS3;
Ile 105 Val (A/G) in the gene encoding GSTP;
Glu 416 Asp (T/G) in the gene encoding VDBP;
Lys 420 Thr (A/C) in the gene encoding VDBP;
-1055 C/T in the promoter of the gene encoding IL13;
the S mutation in the gene encoding α1-antitrypsin;
-308 G/A in the promoter of the gene encoding TNFα;
-511 A/G in the promoter of the gene encoding IL1B;
Tyr 113 His T/C in the gene encoding MEH;
His 139 Arg G/A in the gene encoding MEH;
Gln 27 Glu C/G in the gene encoding ADBR;
-1607 1G/2G in the promoter of the gene encoding MMP1;
-1562 C/T in the promoter of the gene encoding MMP9;
M1 (GSTM1) null in the gene encoding GST-1;
1237 G/A in the 3' region of the gene encoding α1-antitrypsin;
-82 A/G in the promoter of the gene encoding MMP12;
T→C within codon 10 of the gene encoding TGFβ;
760 C/G in the gene encoding SOD3;
-1296 T/C within the promoter of the gene encoding TIMP3; or
the S mutation in the gene encoding α1-antitrypsin.

In another aspect the invention provides a set of nucleotide probes and/or primers for use in the preferred methods of the invention herein described. Preferably, the nucleotide probes and/or primers are those which span, or are able to be used to span, the polymorphic regions of the genes.

In yet a further aspect, the invention provides a nucleic acid microarray for use in the methods of the invention, which microarray comprises a substrate presenting nucleic acid sequences capable of hybridizing to nucleic acid sequences which encode one or more of the susceptibility or protective polymorphisms described herein or sequences complimentary thereto.

In another aspect, the invention provides an antibody microarray for use in the methods of the invention, which microarray comprises a substrate presenting antibodies capable of binding to a product of expression of a gene the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism as described herein.

In a further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema comprising the step of replicating, genotypically or phenotypically, the presence and/or functional effect of a protective polymorphism in said subject.

In yet a further aspect, the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema, said subject having a detectable susceptibility polymorphism which either upregulates or downregulates expression of a gene such that the physiologically active concentration of the expressed gene product is outside a range which is normal for the age and sex of the subject, said method comprising the step of restoring the physiologically active concentration of said product of gene expression to be within a range which is normal for the age and sex of the subject.

In yet a further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the GG genotype at the -765 C/G polymorphism present in the promoter of the gene encoding COX2 has been determined, said method comprising administering to said subject an agent capable of reducing COX2 activity in said subject.

In one embodiment, said agent is a COX2 inhibitor or a nonsteroidal anti-inflammatory drug (NSAID), preferably said COX2 inhibitor is selected from the group consisting of Celebrex (Celecoxib), Bextra (Valdecoxib), and Vioxx (Rofecoxib).

In a further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the AA genotype at the 105 C/A polymorphism in the gene encoding IL18 has been determined, said method comprising administering to said subject an agent capable of augmenting IL18 activity in said subject.

In yet a further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the CC genotype at the -133 G/C polymorphism in the promoter of the gene encoding IL18 has been determined, said method comprising administering to said subject an agent capable of augmenting IL18 activity in said subject.

In still a further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the 5G5G genotype at the -675 4G/5G polymorphism in the promoter of the gene encoding PAI-1 has been determined, said method comprising administering to said subject an agent capable of augmenting PAI-1 activity in said subject.

In a yet further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the AA genotype at the 874 A/T polymorphism in the gene encoding IFN-γ has been determined, said method comprising administering to said subject an agent capable of modulating IFN-γ activity in said subject.

In still yet a further aspect the present invention provides a method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the CC genotype at the -159 C/T polymorphism in the gene encoding CD-14 has been determined, said method comprising administering to said subject an agent capable of modulating CD-14 and/or IgE activity in said subject.

In yet a further aspect, the present invention provides a method for screening for compounds that modulate the expression and/or activity of a gene, the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism, said method comprising the steps of:

contacting a candidate compound with a cell comprising a susceptibility or protective polymorphism which has been determined to be associated with the upregulation or downregulation of expression of a gene; and

measuring the expression of said gene following contact with said candidate compound,

wherein a change in the level of expression after the contacting step as compared to before the contacting step is indicative of the ability of the compound to modulate the expression and/or activity of said gene.

Preferably, said cell is a human lung cell which has been pre-screened to confirm the presence of said polymorphism.

Preferably, said cell comprises a susceptibility polymorphism associated with upregulation of expression of said gene and said screening is for candidate compounds which downregulate expression of said gene.

Alternatively, said cell comprises a susceptibility polymorphism associated with downregulation of expression of said gene and said screening is for candidate compounds which upregulate expression of said gene.

In another embodiment, said cell comprises a protective polymorphism associated with upregulation of expression of said gene and said screening is for candidate compounds which further upregulate expression of said gene.

Alternatively, said cell comprises a protective polymorphism associated with downregulation of expression of said gene and said screening is for candidate compounds which further downregulate expression of said gene.

In another aspect, the present invention provides a method for screening for compounds that modulate the expression and/or activity of a gene, the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism, said method comprising the steps of:

contacting a candidate compound with a cell comprising a gene, the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism but which in said cell the expression of which is neither upregulated nor downregulated; and

measuring the expression of said gene following contact with said candidate compound, wherein a change in the level of expression after the contacting step as compared to before the contacting step is indicative of the ability of the compound to modulate the expression and/or activity of said gene.

Preferably, said cell is human lung cell which has been pre-screened to confirm the presence, and baseline level of expression, of said gene.

Preferably, expression of the gene is downregulated when associated with a susceptibility polymorphism and said screening is for candidate compounds which in said cell, upregulate expression of said gene.

Alternatively, expression of the gene is upregulated when associated with a susceptibility polymorphism and said screening is for candidate compounds which, in said cell, downregulate expression of said gene.

In another embodiment, expression of the gene is upregulated when associated with a protective polymorphism and said screening is for compounds which, in said cell, upregulate expression of said gene.

Alternatively, expression of the gene is downregulated when associated with a protective polymorphism and said screening is for compounds which, in said cell, downregulate expression of said gene.

In yet a further aspect, the present invention provides a method of assessing the likely responsiveness of a subject at risk of developing or suffering from COPD, emphysema, or both COPD and emphysema to a prophylactic or therapeutic treatment, which treatment involves restoring the physiologically active concentration of a product of gene expression to be within a range which is normal for the age and sex of the subject, which method comprises detecting in said subject the presence or absence of a susceptibility polymorphism which when present either upregulates or downregulates expression of said gene such that the physiological active concentration of the expressed gene product is outside said normal range, wherein the detection of the presence of said polymorphism is indicative of the subject likely responding to said treatment.

In a further aspect, the present invention provides a kit for assessing a subject's risk of developing one or more obstructive lung diseases selected from COPD, emphysema, or both COPD and emphysema, said kit comprising a means of analysing a sample from said subject for the presence or absence of one or more polymorphisms disclosed herein.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: depicts a graph showing the percentage of people with COPD plotted against the number of protective genetic variants.

Figure 2: depicts a graph showing the percentage of people with COPD plotted against the number of susceptibility genetic variants.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Using case-control studies the frequencies of several genetic variants (polymorphisms) of candidate genes in smokers who have developed COPD, smokers who appear resistant to COPD, and blood donor controls have been compared. The majority of these candidate genes have confirmed (or likely) functional effects on gene expression or protein function. Specifically the frequencies of polymorphisms between blood donor controls, resistant smokers and those with COPD (subdivided into those with early onset and those with normal onset) have been compared. The present

invention demonstrates that there are both protective and susceptibility polymorphisms present in selected candidate genes of the patients tested.

Specifically, 17 susceptibility genetic polymorphisms and 19 protective genetic polymorphisms have been identified. These are as follows:

Gene	Polymorphism	Role
Cyclo-oxygenase 2 (COX2)	COX2 -765 G/C	CC/CG protective
β2-adrenoreceptor (ADBR)	ADBR Arg16Gly	GG susceptibility
Interleukin -18 (IL18)	IL18 -133 C/G	CC susceptibility
Interleukin -18 (IL18)	IL18 105 A/C	AA susceptibility
Plasminogen activator inhibitor 1 (PAI-1)	PAI-1 -675 4G/5G	5G5G susceptibility
Nitric Oxide synthase 3 (NOS3)	NOS3 298 Asp/Glu	TT protective
Vitamin D Binding Protein (VDBP)	VDBP Lys 420 Thr	AA/AC protective
Vitamin D Binding Protein (VDBP)	VDBP Glu 416 Asp	TT/TG protective
Glutathione S Transferase (GSTP-1)	GSTP1 Ile105Val	AA protective
Interferon γ (IFN-γ)	IFN-γ 874 A/T	AA susceptibility
Interleukin-13 (IL13)	IL13 Arg 130 Gln	AA protective
Interleukin-13 (IL13)	Il13 -1055C/T	TT susceptibility
α1-antitrypsin (α1-AT)	α1-AT S allele	MS protective
Tissue Necrosis Factor α (TNFα)	TNFα +489 G/A	AA/AG susceptibility GG protective
Tissue Necrosis Factor α (TNFα)	TNFα -308 G/A	GG protective AA/AG susceptibility
SMAD3	SMAD3 C89Y AG	AA/AG protective GG susceptibility
Intracellular adhesion molecule 1 (ICAM1)	ICAM1 E469K A/G	GG susceptibility
Caspase (NOD2)	NOD2 Gly 881Arg G/C	GC/CC susceptibility
Mannose binding lectin 2 (MBL2)	MBL2 161 G/A	GG protective
Chymase 1 (CMA1)	CMA1 -1903 G/A	AA protective
N- Acetyl transferase 2 (NAT2)	NAT2 Arg 197 Gln G/A	AA protective
Interleukin 1B (IL1B)	IL1B -511 A/G	GG susceptibility
Microsomal epoxide hydrolase (MEH)	MEH Tyr 113 His T/C	TT susceptibility
Microsomal epoxide hydrolase (MEH)	MEH His 139 Arg G/A	GG protective
5 Lipo-oxygenase (ALOX5)	ALOX5 -366 G/A	AA/AG protective GG susceptibility
Heat Shock Protein 70 (HSP 70)	HSP 70 HOM T2437C	CC/CT susceptibility TT protective
Chloride Channel Calcium-activated 1 (CLCA1)	CLCA1 +13924 T/A	AA susceptibility
Monocyte differentiation antigen CD-14	CD-14 -159 C/T	CC susceptibility

Elafin	Elafin Exon 1 +49 C/T	CT/TT protective
B2-adrenergic receptor (ADBR)	ADBR Gln 27 Glu C/G	GG protective
Matrix metalloproteinase 1 (MMP1)	MMP1 -1607 1G/2G	1G1G/1G2G protective

A susceptibility genetic polymorphism is one which, when present, is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema. In contrast, a protective genetic polymorphism is one which, when present, is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

As used herein, the phrase "risk of developing COPD, emphysema, or both COPD and emphysema" means the likelihood that a subject to whom the risk applies will develop COPD, emphysema, or both COPD and emphysema, and includes predisposition to, and potential onset of the disease. Accordingly, the phrase "increased risk of developing COPD, emphysema, or both COPD and emphysema" means that a subject having such an increased risk possesses an hereditary inclination or tendency to develop COPD, emphysema, or both COPD and emphysema. This does not mean that such a person will actually develop COPD, emphysema, or both COPD and emphysema at any time, merely that he or she has a greater likelihood of developing COPD, emphysema, or both COPD and emphysema compared to the general population of individuals that either does not possess a polymorphism associated with increased COPD, emphysema, or both COPD and emphysema risk, or does possess a polymorphism associated with decreased COPD, emphysema, or both COPD and emphysema risk. Subjects with an increased risk of developing COPD, emphysema, or both COPD and emphysema include those with a predisposition to COPD, emphysema, or both COPD and emphysema, such as a tendency or prediliction regardless of their lung function at the time of assessment, for example, a subject who is genetically inclined to COPD, emphysema, or both COPD and emphysema but who has normal lung function, those at potential risk, including subjects with a tendency to mildly reduced lung function who are likely to go on to suffer COPD, emphysema, or both COPD and emphysema if they keep smoking, and subjects with potential onset of COPD, emphysema, or both COPD and emphysema, who have a tendency to poor lung function on spirometry etc., consistent with COPD at the time of assessment.

Similarly, the phrase "decreased risk of developing COPD, emphysema, or both COPD and emphysema" means that a subject having such a decreased risk possesses an hereditary disinclination or reduced tendency to develop COPD, emphysema, or both

COPD and emphysema. This does not mean that such a person will not develop COPD, emphysema, or both COPD and emphysema at any time, merely that he or she has a decreased likelihood of developing COPD, emphysema, or both COPD and emphysema compared to the general population of individuals that either does possess one or more polymorphisms associated with increased COPD, emphysema, or both COPD and emphysema risk, or does not possess a polymorphism associated with decreased COPD, emphysema, or both COPD and emphysema risk.

It will be understood that in the context of the present invention the term "polymorphism"means the occurrence together in the same population at a rate greater than that attributable to random mutation (usually greater than 1%) of two or more alternate forms (such as alleles or genetic markers) of a chromosomal locus that differ in nucleotide sequence or have variable numbers of repeated nucleotide units. See www.ornl.gov/sci/techresources/Human_Genome/publicat/97pr/09gloss.html#p. Accordingly, the term "polymorphisms" is used herein contemplates genetic variations, including single nucleotide substitutions, insertions and deletions of nucleotides, repetitive sequences (such as microsatellites), and the total or partial absence of genes (eg. null mutations). As used herein, the term "polymorphisms" also includes genotypes and haplotypes. A genotype is the genetic composition at a specific locus or set of loci. A haplotype is a set of closely linked genetic markers present on one chromosome which are not easily separable by recombination, tend to be inherited together, and may be in linkage disequilibrium. A haplotype can be identified by patterns of polymorphisms such as SNPs. Similarly, the term "single nucleotide polymorphism" or "SNP" in the context of the present invention includes single base nucleotide subsitutions and short deletion and insertion polymorphisms.

A reduced or increased risk of a subject developing COPD, emphysema, or both COPD and emphysema may be diagnosed by analysing a sample from said subject for the presence of a polymorphism selected from the group consisting of :

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1;

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding MMP1 (with reference to the 1G allele only);

or one or more polymorphisms which are in linkage disequilibrium with any one or more of the above group.

These polymorphisms can also be analysed in combinations of two or more, or in combination with other polymorphisms indicative of a subject's risk of developing COPD, emphysema, or both COPD and emphysema, inclusive of the remaining polymorphisms listed above.

Expressly contemplated are combinations of the above polymorphisms with polymorphisms as described in PCT International application PCT/NZ02/00106, published as WO 02/099134.

Assays which involve combinations of polymorphisms, including those amenable to high throughput, such as those utilising microarrays, are preferred.

Statistical analyses, particularly of the combined effects of these polymorphisms, show that the genetic analyses of the present invention can be used to determine the risk quotient of any smoker and in particular to identify smokers at greater risk of developing COPD. Such combined analysis can be of combinations of susceptibility polymorphisms only, of protective polymorphisms only, or of combinations of both. Analysis can also be step-wise, with analysis of the presence or absence of protective polymorphisms occurring first and then with analysis of susceptibility polymorphisms proceeding only where no protective polymorphisms are present.

Thus, through systematic analysis of the frequency of these polymorphisms in well defined groups of smokers and non-smokers, as described herein, it is possible to implicate certain proteins in the development of COPD and improve the ability to identify which smokers are at increased risk of developing COPD-related impaired lung function and COPD for predictive purposes.

The present results show for the first time that the minority of smokers who develop COPD, emphysema, or both COPD and emphysema do so because they have one or more of the susceptibility polymorphisms and few or none of the protective polymorphisms defined herein. It is thought that the presence of one or more suscetptible polymorphisms, together with the damaging irritant and oxidant effects of smoking, combine to make this group of smokers highly susceptible to developing COPD, emphysema, or both COPD and emphysema. Additional risk factors, such as familial history, age, weight, pack years, etc., will also have an impact on the risk profile of a subject, and can be assessed in combination with the genetic analyses described herein.

The one or more polymorphisms can be detected directly or by detection of one or more polymorphisms which are in linkage disequilibrium with said one or more polymorphisms. As discussed above, linkage disequilibrium is a phenomenon in genetics whereby two or more mutations or polymorphisms are in such close genetic proximity that they are co-inherited. This means that in genotyping, detection of one polymorphism as present infers the presence of the other. (Reich DE et al; Linkage disequilibrium in the human genome, Nature 2001, 411:199-204.)

Examples of polymorphisms reported to be in linkage disequilibrium are presented herein, and include the Interleukin-18 -133 C/G and 105 A/C polymorphisms, and the Vitamin D binding protein Glu 416 Asp and Lys 420 Thr polymorphisms, as shown below.

Gene	**SNPs**	**rs numbers**	**Alleles in LD**	**LD between alleles**	**Phenotype in COPD**
Interleukin-18	IL18 -133 C/G	rs360721	C allele	Strong LD	CC susceptible
	IL18 105 A/C	rs549908	A allele		AA susceptible
Vitamin D binding protein	VDBP Lys 420 Thr	rs4588	A allele	Strong LD	AA/AC protective
	VDBP Glu 416 Asp	rs7041	T allele		TT/TG protective

It will be apparent that polymorphsisms in linkage disequilibrium with one or more other polymorphism associated with increased or decreased risk of developing

COPD, emphysema, or both COPD and emphysema will also provide utility as biomarkers for risk of developing COPD, emphysema, or both COPD and emphysema. The data presented herein shows that the frequency for SNPs in linkage disequilibrium is very similar. Accordingly, these genetically linked SNPs can be utilized in combined polymorphism analyses to derive a level of risk comparable to that calculated from the original SNP.

It will therefore be apparent that one or more polymorphisms in linkage disequilibrium with the polymorphisms specified herein can be identified, for example, using public data bases. Examples of such polymorphisms reported to be in linkage disequilibrium with the polymorphisms specified herein are presented herein in Table 31.

The methods of the invention are primarily directed to the detection and identification of the above polymorphisms associated with COPD, which are all single nucleotide polymorphisms. In general terms, a single nucleotide polymorphism (SNP) is a single base change or point mutation resulting in genetic variation between individuals. SNPs occur in the human genome approximately once every 100 to 300 bases, and can occur in coding or non-coding regions. Due to the redundancy of the genetic code, a SNP in the coding region may or may not change the amino acid sequence of a protein product. A SNP in a non-coding region can, for example, alter gene expression by, for example, modifying control regions such as promoters, transcription factor binding sites, processing sites, ribosomal binding sites, and affect gene transcription, processing, and translation.

SNPs can facilitate large-scale association genetics studies, and there has recently been great interest in SNP discovery and detection. SNPs show great promise as markers for a number of phenotypic traits (including latent traits), such as for example, disease propensity and severity, wellness propensity, and drug responsiveness including, for example, susceptibility to adverse drug reactions. Knowledge of the association of a particular SNP with a phenotypic trait, coupled with the knowledge of whether an individual has said particular SNP, can enable the targeting of diagnostic, preventative and therapeutic applications to allow better disease management, to enhance understanding of disease states and to ultimately facilitate the discovery of more effective treatments, such as personalised treatment regimens.

Indeed, a number of databases have been constructed of known SNPs, and for some such SNPs, the biological effect associated with a SNP. For example, the NCBI SNP database "dbSNP" is incorporated into NCBI's Entrez system and can be queried using the same approach as the other Entrez databases such as PubMed and GenBank. This database has records for over 1.5 million SNPs mapped onto the human genome sequence. Each dbSNP entry includes the sequence context of the polymorphism (i.e., the surrounding sequence), the occurrence frequency of the polymorphism (by population or individual), and the experimental method(s), protocols, and conditions used to assay the variation, and can include information associating a SNP with a particular phenotypic trait.

At least in part because of the potential impact on health and wellness, there has been and continues to be a great deal of effort to develop methods that reliably and rapidly identify SNPs. This is no trivial task, at least in part because of the complexity of human genomic DNA, with a haploid genome of 3×10^9 base pairs, and the associated sensitivity and discriminatory requirements.

Genotyping approaches to detect SNPs well-known in the art include DNA sequencing, methods that require allele specific hybridization of primers or probes, allele specific incorporation of nucleotides to primers bound close to or adjacent to the polymorphisms (often referred to as "single base extension", or "minisequencing"), allele-specific ligation (joining) of oligonucleotides (ligation chain reaction or ligation padlock probes), allele-specific cleavage of oligonucleotides or PCR products by restriction enzymes (restriction fragment length polymorphisms analysis or RFLP) or chemical or other agents, resolution of allele-dependent differences in electrophoretic or chromatographic mobilities, by structure specific enzymes including invasive structure specific enzymes, or mass spectrometry. Analysis of amino acid variation is also possible where the SNP lies in a coding region and results in an amino acid change.

DNA sequencing allows the direct determination and identification of SNPs. The benefits in specificity and accuracy are generally outweighed for screening purposes by the difficulties inherent in whole genome, or even targeted subgenome, sequencing.

Mini-sequencing involves allowing a primer to hybridize to the DNA sequence adjacent to the SNP site on the test sample under investigation. The primer is extended by one nucleotide using all four differentially tagged fluorescent dideoxynucleotides (A,C,G, or T), and a DNA polymerase. Only one of the four nucleotides (homozygous

case) or two of the four nucleotides (heterozygous case) is incorporated. The base that is incorporated is complementary to the nucleotide at the SNP position.

A number of methods currently used for SNP detection involve site-specific and/or allele-specific hybridisation. These methods are largely reliant on the discriminatory binding of oligonucleotides to target sequences containing the SNP of interest. The techniques of Affymetrix (Santa Clara, Calif.) and Nanogen Inc. (San Diego, Calif.) are particularly well-known, and utilize the fact that DNA duplexes containing single base mismatches are much less stable than duplexes that are perfectly base-paired. The presence of a matched duplex is detected by fluorescence.

The majority of methods to detect or identify SNPs by site-specific hybridisation require target amplification by methods such as PCR to increase sensitivity and specificity (see, for example U.S. Pat. No. 5,679,524, PCT publication WO 98/59066, PCT publication WO 95/12607). US Application 20050059030 (incorporated herein in its entirety) describes a method for detecting a single nucleotide polymorphism in total human DNA without prior amplification or complexity reduction to selectively enrich for the target sequence, and without the aid of any enzymatic reaction. The method utilises a single-step hybridization involving two hybridization events: hybridization of a first portion of the target sequence to a capture probe, and hybridization of a second portion of said target sequence to a detection probe. Both hybridization events happen in the same reaction, and the order in which hybridisation occurs is not critical.

US Application 20050042608 (incorporated herein in its entirety) describes a modification of the method of electrochemical detection of nucleic acid hybridization of Thorp et al. (U.S. Pat. No. 5,871,918). Briefly, capture probes are designed, each of which has a different SNP base and a sequence of probe bases on each side of the SNP base. The probe bases are complementary to the corresponding target sequence adjacent to the SNP site. Each capture probe is immobilized on a different electrode having a non-conductive outer layer on a conductive working surface of a substrate. The extent of hybridization between each capture probe and the nucleic acid target is detected by detecting the oxidation-reduction reaction at each electrode, utilizing a transition metal complex. These differences in the oxidation rates at the different electrodes are used to determine whether the selected nucleic acid target has a single nucleotide polymorphism at the selected SNP site.

The technique of Lynx Therapeutics (Hayward, Calif.) using MEGATYPE^{TM} technology can genotype very large numbers of SNPs simultaneously from small or

large pools of genomic material. This technology uses fluorescently labeled probes and compares the collected genomes of two populations, enabling detection and recovery of DNA fragments spanning SNPs that distinguish the two populations, without requiring prior SNP mapping or knowledge.

A number of other methods for detecting and identifying SNPs exist. These include the use of mass spectrometry, for example, to measure probes that hybridize to the SNP. This technique varies in how rapidly it can be performed, from a few samples per day to a high throughput of 40,000 SNPs per day, using mass code tags. A preferred example is the use of mass spectrometric determination of a nucleic acid sequence which comprises the polymorphisms of the invention, for example, which includes the promoter of the COX2 gene or a complementary sequence. Such mass spectrometric methods are known to those skilled in the art, and the genotyping methods of the invention are amenable to adaptation for the mass spectrometric detection of the polymorphisms of the invention, for example, the COX2 promoter polymorphisms of the invention.

SNPs can also be determined by ligation-bit analysis. This analysis requires two primers that hybridize to a target with a one nucleotide gap between the primers. Each of the four nucleotides is added to a separate reaction mixture containing DNA polymerase, ligase, target DNA and the primers. The polymerase adds a nucleotide to the 3'end of the first primer that is complementary to the SNP, and the ligase then ligates the two adjacent primers together. Upon heating of the sample, if ligation has occurred, the now larger primer will remain hybridized and a signal, for example, fluorescence, can be detected. A further discussion of these methods can be found in U.S. Pat. Nos. 5,919,626; 5,945,283; 5,242,794; and 5,952,174.

US Patent 6,821,733 (incorporated herein in its entirety) describes methods to detect differences in the sequence of two nucleic acid molecules that includes the steps of: contacting two nucleic acids under conditions that allow the formation of a four-way complex and branch migration; contacting the four-way complex with a tracer molecule and a detection molecule under conditions in which the detection molecule is capable of binding the tracer molecule or the four-way complex; and determining binding of the tracer molecule to the detection molecule before and after exposure to the four-way complex. Competition of the four-way complex with the tracer molecule for binding to the detection molecule indicates a difference between the two nucleic acids.

Protein- and proteomics-based approaches are also suitable for polymorphism detection and analysis. Polymorphisms which result in or are associated with variation in expressed proteins can be detected directly by analysing said proteins. This typically requires separation of the various proteins within a sample, by, for example, gel electrophoresis or HPLC, and identification of said proteins or peptides derived therefrom, for example by NMR or protein sequencing such as chemical sequencing or more prevalently mass spectrometry. Proteomic methodologies are well known in the art, and have great potential for automation. For example, integrated systems, such as the ProteomIQ™ system from Proteome Systems, provide high throughput platforms for proteome analysis combining sample preparation, protein separation, image acquisition and analysis, protein processing, mass spectrometry and bioinformatics technologies.

The majority of proteomic methods of protein identification utilise mass spectrometry, including ion trap mass spectrometry, liquid chromatography (LC) and LC/MSn mass spectrometry, gas chromatography (GC) mass spectroscopy, Fourier transform-ion cyclotron resonance-mass spectrometer (FT-MS), MALDI-TOF mass spectrometry, and ESI mass spectrometry, and their derivatives. Mass spectrometric methods are also useful in the determination of post-translational modification of proteins, such as phosphorylation or glycosylation, and thus have utility in determining polymorphisms that result in or are associated with variation in post-translational modifications of proteins.

Associated technologies are also well known, and include, for example, protein processing devices such as the "Chemical Inkjet Printer" comprising piezoelectric printing technology that allows in situ enzymatic or chemical digestion of protein samples electroblotted from 2-D PAGE gels to membranes by jetting the enzyme or chemical directly onto the selected protein spots. After in-situ digestion and incubation of the proteins, the membrane can be placed directly into the mass spectrometer for peptide analysis.

A large number of methods reliant on the conformational variability of nucleic acids have been developed to detect SNPs.

For example, Single Strand Conformational Polymorphism (SSCP, Orita *et al.*, PNAS 1989 86:2766-2770) is a method reliant on the ability of single-stranded nucleic acids to form secondary structure in solution under certain conditions. The secondary structure depends on the base composition and can be altered by a single nucleotide

substitution, causing differences in electrophoretic mobility under nondenaturing conditions. The various polymorphs are typically detected by autoradiography when radioactively labelled, by silver staining of bands, by hybridisation with detectably labelled probe fragments or the use of fluorescent PCR primers which are subsequently detected, for example by an automated DNA sequencer.

Modifications of SSCP are well known in the art, and include the use of differing gel running conditions, such as for example differing temperature, or the addition of additives, and different gel matrices. Other variations on SSCP are well known to the skilled artisan, including,RNA-SSCP, restriction endonuclease fingerprinting-SSCP, dideoxy fingerprinting (a hybrid between dideoxy sequencing and SSCP), bi-directional dideoxy fingerprinting (in which the dideoxy termination reaction is performed simultaneously with two opposing primers), and Fluorescent PCR-SSCP (in which PCR products are internally labelled with multiple fluorescent dyes, may be digested with restriction enzymes, followed by SSCP, and analysed on an automated DNA sequencer able to detect the fluorescent dyes).

Other methods which utilise the varying mobility of different nucleic acid structures include Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), and Heteroduplex Analysis (HET). Here, variation in the dissociation of double stranded DNA (for example, due to base-pair mismatches) results in a change in electrophoretic mobility. These mobility shifts are used to detect nucleotide variations.

Denaturing High Pressure Liquid Chromatography (HPLC) is yet a further method utilised to detect SNPs, using HPLC methods well-known in the art as an alternative to the separation methods described above (such as gel electophoresis) to detect, for example, homoduplexes and heteroduplexes which elute from the HPLC column at different rates, thereby enabling detection of mismatch nucleotides and thus SNPs.

Yet further methods to detect SNPs rely on the differing susceptibility of single stranded and double stranded nucleic acids to cleavage by various agents, including chemical cleavage agents and nucleolytic enzymes. For example, cleavage of mismatches within RNA:DNA heteroduplexes by RNase A, of heteroduplexes by, for example bacteriophage T4 endonuclease YII or T7 endonuclease I, of the 5' end of the hairpin loops at the junction between single stranded and double stranded DNA by cleavase I, and the modification of mispaired nucleotides within heteroduplexes by

chemical agents commonly used in Maxam-Gilbert sequencing chemistry, are all well known in the art.

Further examples include the Protein Translation Test (PTT), used to resolve stop codons generated by variations which lead to a premature termination of translation and to protein products of reduced size, and the use of mismatch binding proteins. Variations are detected by binding of, for example, the MutS protein, a component of *Escherichia coli* DNA mismatch repair system, or the human hMSH2 and GTBP proteins, to double stranded DNA heteroduplexes containing mismatched bases. DNA duplexes are then incubated with the mismatch binding protein, and variations are detected by mobility shift assay. For example, a simple assay is based on the fact that the binding of the mismatch binding protein to the heteroduplex protects the heteroduplex from exonuclease degradation.

Those skilled in the art will know that a particular SNP, particularly when it occurs in a regulatory region of a gene such as a promoter, can be associated with altered expression of a gene. Altered expression of a gene can also result when the SNP is located in the coding region of a protein-encoding gene, for example where the SNP is associated with codons of varying usage and thus with tRNAs of differing abundance. Such altered expression can be determined by methods well known in the art, and can thereby be employed to detect such SNPs. Similarly, where a SNP occurs in the coding region of a gene and results in a non-synonomous amino acid substitution, such substitution can result in a change in the function of the gene product. Similarly, in cases where the gene product is an RNA, such SNPs can result in a change of function in the RNA gene product. Any such change in function, for example as assessed in an activity or functionality assay, can be employed to detect such SNPs.

The above methods of detecting and identifying SNPs are amenable to use in the methods of the invention.

Of course, in order to detect and identify SNPs in accordance with the invention, a sample containing material to be tested is obtained from the subject. The sample can be any sample potentially containing the target SNPs (or target polypeptides, as the case may be) and obtained from any bodily fluid (blood, urine, saliva, etc) biopsies or other tissue preparations.

DNA or RNA can be isolated from the sample according to any of a number of methods well known in the art. For example, methods of purification of nucleic acids are described in Tijssen; Laboratory Techniques in Biochemistry and Molecular

Biology: Hybridization with nucleic acid probes Part 1: Theory and Nucleic acid preparation, Elsevier, New York, N.Y. 1993, as well as in Maniatis, T., Fritsch, E. F. and Sambrook, J., Molecular Cloning Manual 1989.

To assist with detecting the presence or absence of polymorphisms/SNPs, nucleic acid probes and/or primers can be provided. Such probes have nucleic acid sequences specific for chromosomal changes evidencing the presence or absence of the polymorphism and are preferably labeled with a substance that emits a detectable signal when combined with the target polymorphism.

The nucleic acid probes can be genomic DNA or cDNA or mRNA, or any RNA-like or DNA-like material, such as peptide nucleic acids, branched DNAs, and the like. The probes can be sense or antisense polynucleotide probes. Where target polynucleotides are double-stranded, the probes may be either sense or antisense strands. Where the target polynucleotides are single-stranded, the probes are complementary single strands.

The probes can be prepared by a variety of synthetic or enzymatic schemes, which are well known in the art. The probes can be synthesized, in whole or in part, using chemical methods well known in the art (Caruthers et al., *Nucleic Acids Res., Symp. Ser.,* 215-233 (1980)). Alternatively, the probes can be generated, in whole or in part, enzymatically.

Nucleotide analogs can be incorporated into probes by methods well known in the art. The only requirement is that the incorporated nucleotide analog must serve to base pair with target polynucleotide sequences. For example, certain guanine nucleotides can be substituted with hypoxanthine, which base pairs with cytosine residues. However, these base pairs are less stable than those between guanine and cytosine. Alternatively, adenine nucleotides can be substituted with 2,6-diaminopurine, which can form stronger base pairs than those between adenine and thymidine.

Additionally, the probes can include nucleotides that have been derivatized chemically or enzymatically. Typical chemical modifications include derivatization with acyl, alkyl, aryl or amino groups.

The probes can be immobilized on a substrate. Preferred substrates are any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells,

trenches, pins, channels and pores, to which the polynucleotide probes are bound. Preferably, the substrates are optically transparent.

Furthermore, the probes do not have to be directly bound to the substrate, but rather can be bound to the substrate through a linker group. The linker groups are typically about 6 to 50 atoms long to provide exposure to the attached probe. Preferred linker groups include ethylene glycol oligomers, diamines, diacids and the like. Reactive groups on the substrate surface react with one of the terminal portions of the linker to bind the linker to the substrate. The other terminal portion of the linker is then functionalized for binding the probe.

The probes can be attached to a substrate by dispensing reagents for probe synthesis on the substrate surface or by dispensing preformed DNA fragments or clones on the substrate surface. Typical dispensers include a micropipette delivering solution to the substrate with a robotic system to control the position of the micropipette with respect to the substrate. There can be a multiplicity of dispensers so that reagents can be delivered to the reaction regions simultaneously.

Nucleic acid microarrays are preferred. Such microarrays (including nucleic acid chips) are well known in the art (see, for example US Patent Nos 5,578,832; 5,861,242; 6,183,698; 6,287,850; 6,291,183; 6,297,018; 6,306,643; and 6,308,170, each incorporated by reference).

Alternatively, antibody microarrays can be produced. The production of such microarrays is essentially as described in Schweitzer & Kingsmore, "Measuring proteins on microarrays", *Curr Opin Biotechnol* 2002; 13(1): 14-9; Avseekno et al., "Immobilization of proteins in immunochemical microarrays fabricated by electrospray deposition", *Anal Chem* 2001 15; 73(24): 6047-52; Huang, "Detection of multiple proteins in an antibody-based protein microarray system, *Immunol Methods* 2001 1; 255 (1-2): 1-13.

The present invention also contemplates the preparation of kits for use in accordance with the present invention. Suitable kits include various reagents for use in accordance with the present invention in suitable containers and packaging materials, including tubes, vials, and shrink-wrapped and blow-molded packages.

Materials suitable for inclusion in an exemplary kit in accordance with the present invention comprise one or more of the following: gene specific PCR primer pairs (oligonucleotides) that anneal to DNA or cDNA sequence domains that flank the genetic polymorphisms of interest, reagents capable of amplifying a specific sequence

domain in either genomic DNA or cDNA without the requirement of performing PCR; reagents required to discriminate between the various possible alleles in the sequence domains amplified by PCR or non-PCR amplification (e.g., restriction endonucleases, oligonucleotide that anneal preferentially to one allele of the polymorphism, including those modified to contain enzymes or fluorescent chemical groups that amplify the signal from the oligonucleotide and make discrimination of alleles more robust); reagents required to physically separate products derived from the various alleles (e.g. agarose or polyacrylamide and a buffer to be used in electrophoresis, HPLC columns, SSCP gels, formamide gels or a matrix support for MALDI-TOF).

It will be appreciated that the methods of the invention can be performed in conjunction with an analysis of other risk factors known to be associated with COPD, emphysema, or both COPD and emphysema. Such risk factors include epidemiological risk factors associated with an increased risk of developing COPD, emphysema, or both COPD and emphysema. Such risk factors include, but are not limited to smoking and/or exposure to tobacco smoke, age, sex and familial history. These risk factors can be used to augment an analysis of one or more polymorphisms as herein described when assessing a subject's risk of developing chronic obstructive pulmonary disease (COPD) and/or emphysema.

The predictive methods of the invention allow a number of therapeutic interventions and/or treatment regimens to be assessed for suitability and implemented for a given subject. The simplest of these can be the provision to the subject of motivation to implement a lifestyle change, for example, where the subject is a current smoker, the methods of the invention can provide motivation to quit smoking.

The manner of therapeutic intervention or treatment will be predicated by the nature of the polymorphism(s) and the biological effect of said polymorphism(s). For example, where a susceptibility polymorphism is associated with a change in the expression of a gene, intervention or treatment is preferably directed to the restoration of normal expression of said gene, by, for example, administration of an agent capable of modulating the expression of said gene. Where a SNP allele or genotype is associated with decreased expression of a gene, therapy can involve administration of an agent capable of increasing the expression of said gene, and conversely, where a SNP allele or genotype is associated with increased expression of a gene, therapy can involve administration of an agent capable of decreasing the expression of said gene. Methods useful for the modulation of gene expression are well known in the art. For example, in

situations were a SNP allele or genotype is associated with upregulated expression of a gene, therapy utilising, for example, RNAi or antisense methodologies can be implemented to decrease the abundance of mRNA and so decrease the expression of said gene. Alternatively, therapy can involve methods directed to, for example, modulating the activity of the product of said gene, thereby compensating for the abnormal expression of said gene.

Where a susceptibility SNP allele or genotype is associated with decreased gene product function or decreased levels of expression of a gene product, therapeutic intervention or treatment can involve augmenting or replacing of said function, or supplementing the amount of gene product within the subject for example, by administration of said gene product or a functional analogue thereof. For example, where a SNP allele or genotype is associated with decreased enzyme function, therapy can involve administration of active enzyme or an enzyme analogue to the subject. Similarly, where a SNP allele or genotype is associated with increased gene product function, therapeutic intervention or treatment can involve reduction of said function, for example, by administration of an inhibitor of said gene product or an agent capable of decreasing the level of said gene product in the subject. For example, where a SNP allele or genotype is associated with increased enzyme function, therapy can involve administration of an enzyme inhibitor to the subject.

Likewise, when a beneficial (protective) SNP is associated with upregulation of a particular gene or expression of an enzyme or other protein, therapies can be directed to mimic such upregulation or expression in an individual lacking the resistive genotype, and/or delivery of such enzyme or other protein to such individual Further, when a protective SNP is associated with downregulation of a particular gene, or with diminished or eliminated expression of an enzyme or other protein, desirable therapies can be directed to mimicking such conditions in an individual that lacks the protective genotype.

The relationship between the various polymorphisms identified above and the susceptibility (or otherwise) of a subject to COPD, emphysema, or both COPD and emphysema also has application in the design and/or screening of candidate therapeutics. This is particularly the case where the association between a susceptibility or protective polymorphism is manifested by either an upregulation or downregulation of expression of a gene. In such instances, the effect of a candidate therapeutic on such upregulation or downregulation is readily detectable.

For example, in one embodiment existing human lung organ and cell cultures are screened for SNP genotypes as set forth above. (For information on human lung organ and cell cultures, *see, e.g.*: Bohinski et al. (1996) *Molecular and Cellular Biology* **14**:5671-5681; Collettsolberg et al. (1996) *Pediatric Research* **39**:504; Hermanns et al. (2004) *Laboratory Investigation* **84**:736-752; Hume et al. (1996) *In Vitro Cellular & Developmental Biology-Animal* **32**:24-29; Leonardi et al. (1995) **38**:352-355; Notingher et al. (2003) Biopolymers (Biospectroscopy) **72**:230-240; Ohga et al. (1996) *Biochemical and Biophysical Research Communications* **228**:391-396; each of which is hereby incorporated by reference in its entirety.) Cultures representing susceptible and protective genotype groups are selected, together with cultures which are putatively "normal" in terms of the expression of a gene which is either upregulated or downregulated where a protective polymorphism is present.

Samples of such cultures are exposed to a library of candidate therapeutic compounds and screened for any or all of: (a) downregulation of susceptibility genes that are normally upregulated in susceptible genotypes; (b) upregulation of susceptibility genes that are normally downregulated in susceptible genotypes; (c) downregulation of protective genes that are normally downregulated or not expressed (or null forms are expressed) in protective genotypes; and (d) upregulation of protective genes that are normally upregulated in protective genotypes. Compounds are selected for their ability to alter the regulation and/or action of susceptibility genes and/or protective genes in a culture having a susceptible genotype.

Similarly, where the polymorphism is one which when present results in a physiologically active concentration of an expressed gene product outside of the normal range for a subject (adjusted for age and sex), and where there is an available prophylactic or therapeutic approach to restoring levels of that expressed gene product to within the normal range, individual subjects can be screened to determine the likelihood of their benefiting from that restorative approach. Such screening involves detecting the presence or absence of the polymorphism in the subject by any of the methods described herein, with those subjects in which the polymorphism is present being identified as individuals likely to benefit from treatment.

EXAMPLES

The invention will now be described in more detail, with reference to non-limiting examples.

Example 1. Case Association Study

Subject recruitment

Subjects of European descent who had smoked a minimum of fifteen pack years and diagnosed by a physician with chronic obstructive pulmonary disease (COPD) were recruited. Subjects met the following criteria: were over 50 years old and had developed symptoms of breathlessness after 40 years of age, had a Forced expiratory volume in one second (FEV1) as a percentage of predicted <70% and a FEV1/FVC ratio (Forced expiratory volume in one second/Forced vital capacity) of < 79% (measured using American Thoracic Society criteria). Two hundred and ninety-four subjects were recruited, of these 58% were male, the mean FEV1/FVC (± 95%confidence limits) was 51% (49-53), mean FEV1 as a percentage of predicted was 43 (41-45). Mean age, cigarettes per day and pack year history was 65 yrs (64-66), 24 cigarettes/day (22-25) and 50 pack years (41-55) respectively. Two hundred and seventeen European subjects who had smoked a minimum of twenty pack years and who had never suffered breathlessness and had not been diagnosed with an obstructive lung disease in the past, in particular childhood asthma or chronic obstructive lung disease, were also studied. This control group was recruited through clubs for the elderly and consisted of 63% male, the mean FEV1/FVC (95%CI) was 82% (81-83), mean FEV1 as a percentage of predicted was 96 (95-97). Mean age, cigarettes per day and pack year history was 59 yrs (57-61), 24 cigarettes/day (22-26) and 42 pack years (39-45) respectively. Using a PCR based method (Sandford et al., 1999), all subjects were genotyped for the α1-antitrypsin mutations (S and Z alleles) and those with the ZZ allele were excluded. The COPD and resistant smoker cohorts were matched for subjects with the MZ genotype (5% in each cohort). 190 European blood donors (smoking status unknown) were recruited consecutively through local blood donor services. Sixty-three percent were men and their mean age was 50 years. On regression analysis, the age difference and pack years difference observed between COPD sufferers and resistant smokers was found not to determine FEV or COPD.

This study shows that polymorphisms found in greater frequency in COPD patients compared to controls (and/or resistant smokers) can reflect an increased susceptibility to the development of impaired lung function and COPD. Similarly, polymorphisms found in greater frequency in resistant smokers compared to susceptible smokers (COPD patients and/or controls) can reflect a protective role.

Summary of characteristics for the COPD, resistant smoker and healthy blood donors

Parameter Median (IQR)	*COPD N=294*	*Resistant smokers N=217*	*Differences*
% male	58%	63%	ns
Age (yrs)	65 (64-66)	59 (57-61)	P<0.05
Pack years	50 (46-53)	42 (39-45)	P<0.05
Cigarettes/day	24 (22-25)	24(22-26)	ns
FEV1 (L)	1.6 (0.7-2.5)	2.9 (2.8-3.0)	P<0.05
FEV1 % predict	43 (41-45)	96% (95-97)	P<0.05
FEV1/FVC	51 (49-53)	82 (81-83)	P<0.05

Means and 95% confidence limits

Genotyping Methods

Cyclo-oxygenase 2 (COX2) -765 G/C promoter polymorphism and α1-antitrypsin genotyping

Genomic DNA was extracted from whole blood samples (Maniatis,T., Fritsch, E. F. and Sambrook, J., Molecular Cloning Manual. 1989). The Cyclo-oxygenase 2 -765 polymorphism was determined by minor modifications of a previously published method (Papafili A, et al., 2002, incorporated in its entirety herein by reference)). The PCR reaction was carried out in a total volume of 25ul and contained 20 ng genomic DNA, 500pmol forward and reverse primers, 0.2mM dNTPs, 10 mM Tris-HCL (pH 8.4), 150 mM KCl, 1.0 mM $MgCl_2$ and 1 unit of polymerase (Life Technologies). Cycling times were incubations for 3 min at 95°C followed by 33 cycles of 50s at 94°C, 60s at 66°C and 60s at 72°C. A final elongation of 10 min at 72°C then followed. 4ul of PCR products were visualised by ultraviolet trans-illumination of a 3% agarose gel stained with ethidium bromide. An aliquot of 3ul of amplification product was digested for 1 hr with 4 units of *Aci*I (Roche Diagnostics, New Zealand) at 37°C. Digested products were separated on a 2.5% agarose gel run for 2.0 hours at 80 mV with TBE buffer. The products were visualised against a 123bp ladder using ultraviolet transillumination after ethidium bromide staining. Using a PCR based method referenced above (Sandford et al., 1999), all COPD and resistant smoker subjects were genotyped for the α1-antitrypsin S and Z alleles.

Elafin +49C/T polymorphism

Genomic DNA was extracted from whole blood samples (Maniatis,T., Fritsch, E. F. and Sambrook, J., Molecular Cloning Manual. 1989). The Elafin +49 polymorphism was determined by minor modifications of a previously published

method [Kuijpers ALA, et al. Clinical Genetics 1998; 54: 96-101.] incorporated in its entirety herein by reference)). The PCR reaction was carried out in a total volume of 25ul and contained 20 ng genomic DNA, 500pmol forward and reverse primers, 0.2mM dNTPs, 10 mM Tris-HCL (pH 8.4), 150 mM KCl, 1.0 mM $MgCl_2$ and 1 unit of Taq polymerase] (Life Technologies). Cycling times were incubations for 3 min at 95°C followed by 33 cycles of 50s at 94°C, 60s at 66°C and 60s at 72°C. A final elongation of 10 min at 72°C then followed. 4ul of PCR products were visualised by ultraviolet trans-illumination of a 3% agarose gel stained with ethidium bromide. An aliquot of 3ul of amplification product was digested for 1 hr with 4 units of Fok 1 (Roche Diagnostics, New Zealand) at 37°C. Digested products were separated on a 2.5% agarose gel run for 2.0 hours at 80 mV with TBE buffer. The products were visualised against a 123bp ladder using ultraviolet transillumination after ethidium bromide staining.

Genotyping of the -1607 1G2G polymorphism of the matrix metalloproteinase 1 gene

Genomic DNA was extracted using standard phenol and chloroform methods. Cohorts of patients and controls were configured in to 96-well PCR format containing strategic negative controls. The assay primers, PCR conditions and RFLP assays details have been previously described [Dunleavey L, et al]. Genotyping was done using minor modifications of the above protocol optimised for our own laboratory conditions. The PCR reactions were amplified in MJ Research thermocyclers in a total volume of 25µl and contained 80ng genomic DNA, 100 ng forward and reverse primers, 200mM dNTPs, 20 mM Tris-HCL (pH 8.4), 50 mM KCI, 1.5 mM $MgCl_2$ and 1.0 unit of Taq polymerase (Qiagen). Forward and reverse prime sequences were 3' TCG TGA GAA TGT CTT CCC ATT-3' [SEQ ID NO. 1] and 5'TCT TGG ATT GAT TTG AGA TAA GTG AAA TC-3' [SEQ ID NO. 2]. Cycling conditions consisted of 94C 60 s, 55C 30s, 72C 30 s for 35 cycles with an extended last extension of 3 min. Aliquots of amplification product were digested for 4 hrs with 6 Units of the restriction enzymes *XmnI* (Roche Diagnostics, New Zealand) at designated temperature conditions. Digested products were separated on 6% polyacrylamide gel. The products were visualised by ultraviolet transillumination following ethidium bromide staining and migration compared against a 1 Kb plus ladder standard (Invitrogen). Genotypes were recorded in data spreadsheets and statistical analysis performed.

Other polymorphism genotyping

Genomic DNA was extracted from whole blood samples (Maniatis, T., Fritsch, E. F. and Sambrook, J., Molecular Cloning Manual. 1989). Purified genomic DNA was

aliquoted (10 ng/ul concentration) into 96 well plates and genotyped on a Sequenom™ system (Sequenom™ Autoflex Mass Spectrometer and Samsung 24 pin nanodispenser) using the following sequences, amplification conditions and methods.

The following conditions were used for the PCR multiplex reaction: final concentrations were for 10xBuffer 15 mM $MgCl_2$ 1.25x, 25mM $MgCl_2$ 1.625mM, dNTP mix 25 mM 500uM, primers 4 uM 100nM, Taq polymerase (Qiagen hot start) 0.15U/reaction, Genomic DNA 10 ng/ul. Cycling times were 95°C for 15 min, (5°C for 15 s, 56°C 30s, 72°C 30s for 45 cycles with a prolonged extension time of 3min to finish. Shrimp alkaline phosphotase (SAP) treatment was used (2ul to 5ul per PCR reaction) incubated at 35°C for 30 min and extension reaction (add 2ul to 7ul after SAP treatment) with the following volumes per reaction of: water, 0.76ul; hME 10x termination buffer, 0.2ul; hME primer (10uM), 1ul; MassEXTEND enzyme, 0.04ul.

Sequenom conditions for the polymorphisms genotyping -1

SNP_ID	TERM	WELL	2nd-PCRP	1st-PCRP
Vitamin DBP - 420	ACT	W1	ACGTTGGATGGCTTGTTAACCAGCTTTGCC[SEQ.ID.NO.3]	ACGTTGGATGTTTTTCAGACTGGCAGAGCG[SEQ.ID.NO.4]
Vitamin DBP - 416	ACT	W1	ACGTTGGATGTTTTTCAGACTGGCAGAGCG[SEQ.ID.NO.5]	ACGTTGGATGGCTTGTTAACCAGCTTTGCC[SEQ.ID.NO.6]
IL13 C-1055T	ACT	W2	ACGTTGGATGCATGTCGCCTTTTCCTGCTC[SEQ.ID.NO.7]	ACGTTGGATGCAACACCCAACAGGCAAATG[SEQ.ID.NO.8]
GSTP1 - 105	ACT	W2	ACGTTGGATGTGGTGGACATGGTGAATGAC[SEQ.ID.NO.9]	ACGTTGGATGTGGTGCAGATGCTCACATAG[SEQ.ID.NO.10]
PAI1 G-675G	ACT	W2	ACGTTGGATGCACAGAGAGAGTCTGGACAC[SEQ.ID.NO.11]	ACGTTGGATGCTCTTGGTCTTTCCCTCATC[SEQ.ID.NO.12]
NOS3 -298	ACT	W3	ACGTTGGATGACAGCTCTGCATTCAGCACG[SEQ.ID.NO.13]	ACGTTGGATGAGTCAATCCCTTTGGTGCTC[SEQ.ID.NO.14]
IL13-Arg130Gln	ACT	W3	ACGTTGGATGGTTTTCCAGCTTGCATGTCC[SEQ.ID.NO.15]	ACGTTGGATGCAATAGTCAGGTCCTGTCTC[SEQ.ID.NO.16]
ADRB2-Arg16Gly	ACT	W3	ACGTTGGATGGAACGGCAGCGCCTTCTTG[SEQ.ID.NO.17]	ACGTTGGATGACTTGGCAATGGCTGTGATG[SEQ.ID.NO.18]
IFNG - A874T	CGT	W5	ACGTTGGATGCAGACATTCACAATTGATTT[SEQ.ID.NO.19]	ACGTTGGATGGATAGTTCCAAACATGTGCG[SEQ.ID.NO.20]
IL18- C-133G	ACT	W6	ACGTTGGATGGGGTATTCATAAGCTGAAAC[SEQ.ID.NO.21]	ACGTTGGATGCCTTCAAGTTCAGTGGTCAG[SEQ.ID.NO.22]
IL18- A105C	ACT	W8	ACGTTGGATGGGTCAATGAAGAGAACTTGG[SEQ.ID.NO.23]	ACGTTGGATGAATGTTTATTGTAGAAAACC[SEQ.ID.NO.24]

Sequenom conditions for the polymorphisms genotyping -2

SNP_ID	AMP_LEN	UP_CONF	MP_CONF	Tm(NN)	PcGC	PWARN	UEP_DIR
Vitamin DBP - 420	99	99.7	99.7	46.2	53.3	ML	R
Vitamin DBP - 416	99	99.7	99.7	45.5	33.3	M	F
IL13 C-1055T	112	97.5	80	48.2	60	L	R
GSTP1 - 105	107	99.4	80	49.9	52.9		F
PAI1 G-675G	109	97.9	80	59.3	66.7	g	F
NOS3 -298	186	98.1	65	61.2	63.2		F
IL13-Arg130Gln	171	99.3	65	55.1	47.6		F
ADRB2- Arg16Gly	187	88.2	65	65.1	58.3		F
IFNG - A874T	112	75.3	81.2	45.6	27.3		F
IL18- C-133G	112	93.5	74.3	41.8	46.7	L	F
IL18- A105C	121	67.2	74.3	48.9	40		R

Sequenom conditions for the polymorphisms genotyping -3

SNP_ID	UEP_MASS	UEP_SEQ	EXT1_CALL	EXT1_MASS
Vitamin DBP - 420	4518.9	AGCTTTGCCAGTTCC [SEQ ID NO. 25]	A	4807.1
Vitamin DBP - 416	5524.6	AAAAGCAAAATTGCCTGA [SEQ ID NO. 26]	T	5812.8
IL13 C-1055T	4405.9	TCCTGCTCTTCCCTC [SEQ ID NO. 27]	T	4703.1
GSTP1 - 105	5099.3	ACCTCCGCTGCAAATAC [SEQ ID NO. 28]	A	5396.5
PAI1 G-675G	5620.6	GAGTCTGGACACGTGGGG [SEQ ID NO. 29]	DEL	5917.9
NOS3 -298	5813.8	TGCTGCAGGCCCCAGATGA [SEQ ID NO. 30]	T	6102
IL13-Arg130Gln	6470.2	AGAAACTTTTTCGCGAGGGAC [SEQ ID NO. 31]	A	6767.4
ADRB2- Arg16Gly	7264.7	AGCGCCTTCTTGCTGGCACCCAAT [SEQ ID NO. 32]	A	7561.9
IFNG - A874T	6639.4	TCTTACAACACAAAATCAAATC [SEQ ID NO. 33]	T	6927.6
IL18- C-133G	4592	AGCTGAAACTTCTGG [SEQ ID NO. 34]	C	4865.2
IL18- A105C	6085	TCAAGCTTGCCAAAGTAATC [SEQ ID NO. 35]	A	6373.2

Sequenom conditions for the polymorphisms genotyping -4

SNP_ID	EXT1_SEQ	EXT2_ CALL	EXT2_ MASS	EXT2_SEQ	1st PAUSE
VitaminDBP-420	AGCTTTGCCAGTTCCT[SEQ.ID.NO.36]	C	5136.4	AGCTTTGCCAGTTCCGT[SEQ.ID.NO.37]	4848.2
VitaminDBP-416	AAAAGCAAAATTGCCTGAT[SEQ.ID.NO.38]	G	6456.2	AAAAGCAAAATTGCCTGAGGC[SEQ.ID.NO.39]	5853.9
IL13C-1055T	TCCTGCTCTTCCCTCA[SEQ.ID.NO.40]	C	5023.3	TCCTGCTCTTCCCTCGT[SEQ.ID.NO.41]	4735.1
GSTP1-105	ACCTCCGCTGCAAATACA[SEQ.ID.NO.42]	G	5716.7	ACCTCCGCTGCAAATACGT[SEQ.ID.NO.43]	5428.5
PAI1G-675G	GAGTCTGGACACGTGGGGA[SEQ.ID.NO.44]	G	6247.1	GAGTCTGGACACGTGGGGGA[SEQ.ID.NO.45]	5949.9
NOS3-298	TGCTGCAGGCCCCAGATGAT[SEQ.ID.NO.46]	G	6416.2	TGCTGCAGGCCCCAGATGAGC[SEQ.ID.NO.47]	6143
IL13-Arg130Gln	AGAAACTTTTTCGCGAGGGACA[SEQ.ID.NO.48]	G	7416.8	AGAAACTTTTTCGCGAGGGACGGT[SEQ.ID.NO.49]	6799.4
ADRB2-Arg16Gly	AGCGCCTTCTTGCTGGCACCCAATA[SEQ.ID.NO. 50]	G	8220.3	AGCGCCTTCTTGCTGGCACCCAATGGA[SEQ.ID. NO. 51]	7593.9
IFNG-A874T	TCTTACAACACAAAATCAAATCT[SEQ.ID.NO.52]	A	7225.8	TCTTACAACACAAAATCAAATCAC[SEQ.ID.NO.53]	6952.6
IL18-C-133G	AGCTGAAACTTCTGGC[SEQIDNO.54]	G	5218.4	AGCTGAAACTTCTGGGA[SEQ.ID.NO.55]	4921.2
IL18-A105C	TCAAGCTTGCCAAAGTAATCT[SEQ.ID.NO.56]	C	7040.6	TCAAGCTTGCCAAAGTAATCGGA[SEQ.ID.NO.57]	6414.2

Sequenom conditions for the polymorphisms genotyping-5

SNP_ID	2nd-PCRP	1st-PCRP
Lipoxygenase5-366G/A	ACGTTGGATGGAAGTCAGAGATGATGGCAG [SEQ.ID.NO.58]	ACGTTGGATGATGAATCCTGGACCCAAGAC [SEQ.ID.NO.59]
TNFalpha+489G/A	ACGTTGGATGGAAAGATGTGCGCTGATAGG [SEQ.ID.NO.60]	ACGTTGGATGGCCACATCTCTTTCTGCATC [SEQ.ID.NO.61]
SMAD3C89Y	ACGTTGGATGTTGCAGGTGTCCCATCGGAA [SEQ.ID.NO.62]	ACGTTGGATGTAGCTCGTGGTGGCTGTGCA [SEQ.ID.NO.63]
CaspaseGly881ArgG/C	ACGTTGGATGGTGATCACCCAAGGCTTCAG [SEQ.ID.NO.64]	ACGTTGGATGGTCTGTTGACTCTTTTGGCC [SEQ.ID.NO.65]
MBL2+161G/A	ACGTTGGATGGTAGCTCTCCAGGCATCAAC [SEQ.ID.NO.66]	ACGTTGGATGGTACCTGGTTCCCCCTTTTC [SEQ.ID.NO.67]
HSP70-HOM2437T/C	ACGTTGGATGTGATCTTGTTCACCTTGCCG [SEQ.ID.NO.68]	ACGTTGGATGAGATCGAGGTGACGTTTGAC [SEQ.ID.NO.69]
CD14-159C/T	ACGTTGGATGAGACACAGAACCCTAGATGC [SEQ.ID.NO.70]	ACGTTGGATGGCAATGAAGGATGTTTCAGG [SEQ.ID.NO.71]
Chymase1-1903G/A	ACGTTGGATGTAAGACAGCTCCACAGCATC [SEQ.ID.NO.72]	ACGTTGGATGTTCCATTTCCTCACCCTCAG [SEQ.ID.NO.73]
TNFalpha-308G/A	ACGTTGGATGGATTTGTGTGTAGGACCCTG [SEQ.ID.NO.74]	ACGTTGGATGGGTCCCCAAAAGAAATGGAG [SEQ.ID.NO.75]
CLCA1+13924T/A	ACGTTGGATGGGATTGGAGAACAAACTCAC [SEQ.ID.NO.76]	ACGTTGGATGGGCAGCTGTTACACCAAAAG [SEQ.ID.NO.77]
MEHTyr113HisT/C	ACGTTGGATGCTGGCGTTTTGCAAACATAC [SEQ.ID.NO.78]	ACGTTGGATGTTGACTGGAAGAAGCAGGTG [SEQ.ID.NO.79]
NAT2Arg197GlnG/A	ACGTTGGATGCCTGCCAAAGAAGAAACACC [SEQ.ID.NO.80]	ACGTTGGATGACGTCTGCAGGTATGTATTC [SEQ.ID.NO.81]
MEHHis139ArgG/A	ACGTTGGATGACTTCATCCACGTGAAGCCC [SEQ.ID.NO.82]	ACGTTGGATGAAACTCGTAGAAAGAGCCGG [SEQ.ID.NO.83]
IL-1B-511A/G	ACGTTGGATGATTTTCTCCTCAGAGGCTCC [SEQ.ID.NO.84]	ACGTTGGATGTGTCTGTATTGAGGGTGTGG [SEQ.ID.NO.85]
ADRB2Gln27GluC/G	ACGTTGGATGTTGCTGGCACCCAATGGAAG [SEQ.ID.NO.86]	ACGTTGGATGATGAGAGACATGACGATGCC [SEQ.ID.NO.87]
ICAM1E469KA/G	ACGTTGGATGACTCACAGAGCACATTCACG [SEQ.ID.NO.88]	ACGTTGGATGTGTCACTCGAGATCTTGAGG [SEQ.ID.NO.89]

Sequenom conditions for the polymorphisms genotyping-6

SNP_ID	AMP_LEN	UP_CONF	MP_CONF	Tm(NN)	PcGC	UEP_DIR
Lipoxygenase5-366G/A	104	99.6	73.4	59	70.6	F
TNFalpha+489G/A	96	99.6	73.4	45.5	38.9	F
SMAD3C89Y	107	87.3	71.7	45.7	47.1	F
CaspaseGly881ArgG/C	111	97.2	81	52.9	58.8	R
MBL2+161G/A	99	96.8	81	50.3	52.9	F
HSP70-HOM2437T/C	107	99.3	81	62.2	65	R
CD14-159C/T	92	98	76.7	53.3	50	F
Chymase1-1903G/A	105	99.6	76.7	53.6	39.1	R
TNFalpha-308G/A	100	99.7	81.6	59.9	70.6	R
CLCA1+13924T/A	101	98	98	45.3	36.8	R
MEHTyr113HisT/C	103	97.7	82.2	48.7	42.1	R
NAT2Arg197GlnG/A	115	97.4	70	48.5	36.4	F
MEHHis139ArgG/A	115	96.7	77.8	66	82.4	F

IL-1B-511A/G	111	99.2	83	46	47.1	R
ADRB2Gln27GluC/G	118	96.6	80	52.2	66.7	F
ICAM1E469KA/G	115	98.8	95.8	51.5	52.9	R

Sequenom conditions for the polymorphisms genotyping-7

SNP_ID	UEP_MASS	UEP_SEQ	EXT1_CALL	EXT1_MASS
Lipoxygenase5-366G/A	5209.4	GTGCCTGTGCTGGGCTC [SEQ.ID.NO.90]	A	5506.6
TNFalpha+489G/A	5638.7	GGATGGAGAGAAAAAAAC [SEQ.ID.NO.91]	A	5935.9
SMAD3C89Y	5056.3	CCCTCATGTCATCTACT [SEQ.ID.NO.92]	A	5353.5
CaspaseGly881ArgG/C	5097.3	GTCACCCACTCTGTTGC [SEQ.ID.NO.93]	G	5370.5
MBL2+161G/A	5299.5	CAAAGATGGGCGTGATG [SEQ.ID.NO.94]	A	5596.7
HSP70-HOM2437T/C	6026.9	CCTTGCCGGTGCTCTTGTCC [SEQ.ID.NO.95]	T	6324.1
CD14-159C/T	6068	CAGAATCCTTCCTGTTACGG [SEQ.ID.NO.96]	C	6341.1
Chymase1-1903G/A	6973.6	TCCACCAAGACTTAAGTTTTGCT [SEQ.ID.NO.97]	G	7246.7
TNFalpha-308G/A	5156.4	GAGGCTGAACCCCGTCC [SEQ.ID.NO.98]	G	5429.5
CLCA1+13924T/A	5759.8	CTTTTTCATAGAGTCCTGT [SEQ.ID.NO.99]	A	6048
MEHTyr113HisT/C	5913.9	TTAGTCTTGAAGTGAGGGT [SEQ.ID.NO.100]	T	6211.1
NAT2Arg197GlnG/A	6635.3	TACTTATTTACGCTTGAACCTC [SEQ.ID.NO.101]	A	6932.5
MEHHis139ArgG/A	5117.3	CCAGCTGCCCGCAGGCC [SEQ.ID.NO.102]	A	5414.5
IL-1B-511A/G	5203.4	AATTGACAGAGAGCTCC [SEQ.ID.NO.103]	G	5476.6
ADRB2Gln27GluC/G	4547	CACGACGTCACGCAG [SEQ.ID.NO.104]	C	4820.2
ICAM1E469KA/G	5090.3	CACATTCACGGTCACCT [SEQ.ID.NO.105]	G	5363.5

Sequenom conditions for the polymorphisms genotyping-8

SNP_ID	EXT1_SEQ	EXT2 CALL	EXT2 MASS	EXT2_SEQ	1st PAUSE
Lipoxygenase5-366G/A	GTGCCTGTGCTGGGCTCA [SEQ.ID.NO.106]	G	5826.8	GTGCCTGTGCTGGGCTCGT [SEQ.ID.NO.107]	5538.6
TNFalpha+489G/A	GGATGGAGAGAAAAAAACA [SEQ.ID.NO.108]	G	6256.1	GGATGGAGAGAAAAAAACGT [SEQ.ID.NO.109]	5967.9
SMAD3C89Y	CCCTCATGTCATCTACTA [SEQ.ID.NO.110]	G	5658.7	CCCTCATGTCATCTACTGC [SEQ.ID.NO.111]	5385.5
CaspaseGly881ArgG/C	GTCACCCACTCTGTTGCC [SEQ.ID.NO.112]	C	5699.7	GTCACCCACTCTGTTGCGC [SEQ.ID.NO.113]	5426.5
MBL2+161G/A	CAAAGATGGGCGTGATGA [SEQ.ID.NO.114]	G	5901.9	CAAAGATGGGCGTGATGGC [SEQ.ID.NO.115]	5628.7
HSP70-HOM2437T/C	CCTTGCCGGTGCTCTTGTCCA [SEQ.ID.NO.116]	C	6644.3	CCTTGCCGGTGCTCTTGTCCGT [SEQ.ID.NO.117]	6356.1
CD14-159C/T	CAGAATCCTTCCTGTTACGGC [SEQ.ID.NO.118]	T	6645.3	CAGAATCCTTCCTGTTACGGTC [SEQ.ID.NO.119]	6372.2
Chymase1-1903G/A	TCCACCAAGACTTAAGTTTTGCTC[SEQ.ID.NO.120]	A	7550.9	TCCACCAAGACTTAAGTTTTGCTTC[SEQ.ID.NO.121]	7277.8
TNFalpha-308G/A	GAGGCTGAACCCCGTCCC [SEQ.ID.NO.122]	A	5733.7	GAGGCTGAACCCCGTCCTC [SEQ.ID.NO.123]	5460.6
CLCA1+13924T/A	CTTTTTCATAGAGTCCTGTT [SEQ.ID.NO.124]	T	6659.4	CTTTTTCATAGAGTCCTGTAAC [SEQ.ID.NO.125]	6073
MEHTyr113HisT/C	TTAGTCTTGAAGTGAGGGTA [SEQ.ID.NO.126]	C	6531.3	TTAGTCTTGAAGTGAGGGTGT [SEQ.ID.NO.127]	6243.1
NAT2Arg197GlnG/A	TACTTATTTACGCTTGAACCTCA [SEQ.ID.NO.128]	G	7261.8	TACTTATTTACGCTTGAACCTCGA [SEQ.ID.NO.129]	6964.5
MEHHis139ArgG/A	CCAGCTGCCCGCAGGCCA [SEQ.ID.NO.130]	G	5734.7	CCAGCTGCCCGCAGGCCGT [SEQ.ID.NO.131]	5446.5
IL-1B-511A/G	AATTGACAGAGAGCTCCC [SEQ.ID.NO.132]	A	5820.8	AATTGACAGAGAGCTCCTG [SEQ.ID.NO.133]	5507.6
ADRB2Gln27GluC/G	CACGACGTCACGCAGC [SEQ.ID.NO.134]	G	5173.4	CACGACGTCACGCAGGA [SEQ.ID.NO.135]	4876.2
ICAM1E469KA/G	CACATTCACGGTCACCTC [SEQ.ID.NO.136]	A	5707.7	CACATTCACGGTCACCTTG [SEQ.ID.NO.137]	5394.5

Results

Table 1. Cyclo-oxygenase 2 -765 G/C polymorphism allele and genotype frequency in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	G	CC	CG	GG
Controls n=94 (%)	27 (14%)	161 (86%)	3 (3%)	21 (22%)	70 (75%)
COPD n=202 (%)	59 (15%)	345 (85%)	6 (3%)	47 (23%)	149[1] (74%)
Resistant n=172 (%)	85[2] (25%)	259 (75%)	14 (8%)	57[1] (33%)	101[1] (59%)

* number of chromosomes (2n)Genotype

1. Genotype. CC/CG vs GG for resistant vs COPD, Odds ratio (OR) =1.98, 95% confidence limits 1.3-3.1, χ^2 (Yates corrected)= 8.82, p=0.003,
   CC/CG = protective for COPD
2. Allele. C vs G for resistant vs COPD, Odds ratio (OR) =1.92, 95% confidence limits 1. 3-2.8, χ^2 (Yates corrected)= 11.56, p<0.001,
   C = protective for COPD

Table 2. Beta2-adrenoreceptor Arg 16 Gly polymorphism allele and genotype frequency in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Controls n=182 (%)	152 (42%)	212 (58%)	26 (14%)	100 (55%)	56 (31%)
COPD n=236 (%)	164 (34%)	308 (66%)	34 (14%)	96 (41%)	106[1] (45%)
Resistant n=190 (%)	135 (36%)	245 (64%)	34 (18%)	67 (35%)	89[2] (47%)

* number of chromosomes (2n)

1. Genotype. GG vs AG/AA for COPD vs controls, Odds ratio (OR) =1.83, 95% confidence limits 1.2-2.8, χ^2 (Yates corrected)= 8.1, p=0.004,
   GG = susceptible to COPD(depending on the presence of other snps)
2. Genotype. GG vs AG/AA for resistant vs controls, Odds ratio (OR) =1.98, 95% confidence limits 1.3-3.1, χ^2 (Yates corrected)=9.43, p=0.002
   GG = protective for COPD (depending on the presence of other snps)

Table 3a. Interleukin 18 105 A/C polymorphism allele and genotype frequency in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	A	CC	AC	AA
Controls n=184 (%)	118 (32%)	250 (68%)	22 (12%)	74 (40%)	88 (48%)
COPD n=240 (%)	122 (25%)	377[2] (75%)	21 (9%)	80 (33%)	139[1,3] (58%)
Resistant n=196 (%)	113 (29%)	277 (71%)	16 (8%)	81 (41%)	99 (50%)

* number of chromosomes (2n)

1. Genotype. AA vs AC/CC for COPD vs controls, Odds ratio (OR) =1.50, 95% confidence limits 1.0-2.3, χ^2 (Yates uncorrected)= 4.26, p=0.04,
   AA = susceptible to COPD
2. Allele. A vs C for COPD vs control, Odds ratio (OR) =1.46, 95% confidence limits 1.1-2.0, χ^2 (Yates corrected)= 5.76, p=0.02
3. Genotype. AA vs AC/CC for COPD vs resistant, Odds ratio (OR) =1.35, 95% confidence limits 0.9-2.0, χ^2 (Yates uncorrected)=2.39, p=0.12 (trend)
   AA = susceptible to COPD

Table 3b. Interleukin 18 -133 C/G polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	G	C	GG	GC	CC
Controls n=187 (%)	120 (32%)	254 (68%)	23 (12%)	74 (40%)	90 (48%)
COPD n=238	123 (26%)	353[2] (74%)	21 (9%)	81 (34%)	136[1] (57%)
Resistant n=195 (%)	113 (29%)	277 (71%)	16 (8%)	81 (42%)	98 (50%)

* number of chromosomes (2n)

1. Genotype. CC vs CG/GG for COPD vs controls, Odds ratio (OR) =1.44, 95% confidence limits 1.0-2.2, χ^2 (Yates corrected)= 3.4, p=0.06,
   CC = susceptible to COPD
2. Allele. C vs G for COPD vs control, Odds ratio (OR) =1.36, 95% confidence limits 1.0-1.9, χ^2 (Yates corrected)= 53.7, p=0.05
   C = susceptible to COPD

Table 4. Plasminogen activator inhibitor 1 -675 4G/5G promoter polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	5G	4G	5G5G	5G4G	4G4G
Controls n=186 (%)	158 (42%)	214 (58%)	31 (17%)	96 (52%)	59 (32%)
COPD n=237 (%)	219[3] (46%)	255 (54%)	54[1,2] (23%)	111 (47%)	72 (30%)
Resistant n=194 (%)	152 (39%)	236 (61%)	31 (16%)	90 (46%)	73[1,2] (38%)

* number of chromosomes (2n)

1. Genotype. 5G5G vs rest for COPD vs resistant, Odds ratio (OR) =1.55, 95% confidence limits 0.9-2.6, χ^2 (Yates uncorrected)= 3.12, p=0.08,
   5G5G = susceptible to COPD
2. Genotype. 5G5G vs rest for COPD vs control, Odds ratio (OR) =1.48, 95% confidence limits 0.9-2.5, χ^2 (Yates uncorrected)= 2.43, p=0.12
   5G5G = susceptible to COPD
3. Allele. 5G vs 4G for COPD vs resistant, Odds ratio (OR) =1.33, 95% confidence limits 1.0-1.8, χ^2 (Yates corrected)=4.02, p=0.05
   5G = susceptible to COPD

Table 5. Nitric oxide synthase 3 Asp 298 Glu (T/G) polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	T	G	TT	TG	GG
Controls n=183 (%)	108 (30%)	258 (70%)	13 (7%)	82 (45%)	88 (48%)
COPD n=238 (%)	159 (42%)	317 (58%)	25 (10%)	109 (47%)	104 (43%)
Resistant n=194 (%)	136 (35%)	252 (65%)	28[1] (15%)	80 (41%)	86 (44%)

* number of chromosomes (2n)

1. Genotype. TT vs TG/GG for resistant vs controls, Odds ratio (OR) =2.2, 95% confidence limits 1.0-4.7, χ^2 (Yates corrected)= 4.49, p=0.03,
   TT genotype = protective for COPD

Table 6a. Vitamin D Binding Protein Lys 420 Thr (A/C) polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	C	AA	AC	CC
Controls n=189 (%)	113 (30%)	265 (70%)	17 (9%)	79 (42%)	93 (49%)
COPD n=250 (%)	147 (29%)	353 (71%)	24 (10%)	99 (40%)	127 (50%)
Resistant n=195 (%)	140[2] (36%)	250 (64%)	25[1] (13%)	90[1] (46%)	80 (41%)

* number of chromosomes (2n)

1. Genotype. AA/AC vs CC for resistant vs COPD, Odds ratio (OR) =1.39, 95% confidence limits 0.9-2.1, χ^2 (Yates uncorrected)= 2.59, p=0.10,
   AA/AC genotype = protective for COPD
2. Allele. A vs C for resistant vs COPD, Odds ratio (OR) =1.34, 95% confidence limits 1.0-1.8, χ^2 (Yates corrected)=3.94, p=0.05
   A allele = protective for COPD

Table 6b. Vitamin D Binding Protein Glu 416 Asp (T/G) polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	T	G	TT	TG	GG
Controls n=188 (%)	162 (43%)	214 (57%)	35 (19%)	92 (49%)	61 (32%)
COPD n=240 (%)	230 (48%)	250 (52%)	57 (24%)	116 (48%)	67 (28%)
Resistant n=197 (%)	193[2] (49%)	201 (51%)	43[1] (22%)	107[1] (54%)	47 (24%)

* number of chromosomes (2n)

1. Genotype. TT/TG vs GG for resistant vs controls, Odds ratio (OR) =1.53, 95% confidence limits 1.0-2.5, χ^2 (Yates uncorrected)= 3.52, p=0.06,
   TT/TG genotype = protective for COPD
2. Allele. T vs G for resistant vs control, Odds ratio (OR) =1.27, 95% confidence limits 1.0-1.7, χ^2 (Yates corrected)=2.69, p=0.1
   T allele = protective for COPD

Table 7. Glutathione S Transferase P1 Ile 105 Val (A/G) polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Controls n=185 (%)	232 (63%)	138 (37%)	70 (38%)	92 (50%)	23 (12%)
COPD n=238 (%)	310 (65%)	166 (35%)	96 (40%)	118 (50%)	24 (10%)
Resistant n=194 (%)	269[2] (69%)	119 (31%)	91[1] (47%)	87 (45%)	16 (8%)

* number of chromosomes (2n)

1. Genotype. AA vs AG/GG for resistant vs controls, Odds ratio (OR) =1.45, 95% confidence limits 0.9-2.2, χ^2 (Yates uncorrected)= 3.19, p=0.07,
   AA genotype = protective for COPD
2. Allele. A vs G for resistant vs control, Odds ratio (OR) =1.34, 95% confidence limits 1.0-1.8, χ^2 (Yates uncorrected)=3.71, p=0.05
   A allele = protective for COPD

Table 8. Interferon-gamma 874 A/T polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	T	AA	AT	TT
Controls n=186 (%)	183 (49%)	189 (51%)	37 (20%)	109 (58%)	40 (22%)
COPD n=235 (%)	244 (52%)	226 (48%)	64[1] (27%)	116 (49%)	55 (24%)
Resistant n=193 (%)	208 (54%)	178 (46%)	51 (27%)	106 (55%)	36 (18%)

* number of chromosomes (2n)

1. Genotype. AA vs AT/TT for COPD vs controls, Odds ratio (OR) =1.51, 95% confidence limits 0.9-2.5, χ^2 (Yates uncorrected)= 3.07, p=0.08,
   AA genotype = susceptible to COPD

Table 9a. Interleukin-13 Arg 130 Gln (G/A) polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Controls n=184 (%)	67 (18%)	301 (82%)	3 (2%)	61 (33%)	120 (65%)
COPD n=237 (%)	86 (18%)	388 (82%)	8 (3%)	70 (30%)	159 (67%)
Resistant n=194 (%)	74 (19%)	314 (81%)	9[1] (5%)	56 (28%)	129 (67%)

* number of chromosomes (2n)

1. Genotype. AA vs AG/GG for resistant vs controls, Odds ratio (OR) =2.94, 95% confidence limits 0.7-14.0, χ^2 (Yates uncorrected)= 2.78, p=0.09,
   AA genotype = protective for COPD

Table 9b. Interleukin-13 -1055 C/T promoter polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	T	C	TT	TC	CC
Controls n=182 (%)	65 (18%)	299 (82%)	5 (3%)	55 (30%)	122 (67%)
COPD n=234 (%)	94 (20%)	374 (80%)	8[1] (4%)	78 (33%)	148 (63%)
Resistant n=192 (%)	72 (19%)	312 (81%)	2 (1%)	68 (35%)	122 (64%)

* number of chromosomes (2n)

1. Genotype. TT vs TC/CC for COPD vs resistant, Odds ratio (OR) =6.03, 95% confidence limits 1.1-42, χ^2 (Yates corrected)= 4.9, p=0.03,
   TT = susceptible to COPD

Table 10. α1-antitrypsin S polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	M	S	MM	MS	SS
COPD n=202 (%)	391 (97%)	13 (3%)	189 (94%)	13 (6%)	0 (0%)
Resistant n=189 (%)	350 (93%)	28 (7%)	162 (85%)	26[1] (14%)	1[1] (1%)

* number of chromosomes (2n)

1. Genotype. MS/SS vs MM for Resistant vs COPD, Odds ratio (OR) =2.42, 95% confidence limits 1.2-5.1, χ^2 (Yates corrected)= 5.7, p=0.01,
   S= protective for COPD

Table 11a. Tissue Necrosis Factor α +489 G/A polymorphism allele and genotype frequency in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=242 (%)	54 (11%)	430 (89%)	5 (2%)	44 (18%)	193 (80%)
Resistant n=187 (%)	27 (7%)	347 (93%)	1 (1%)	25 (13%)	161 (86%)

* number of chromosomes (2n)

1. Genotype. AA/AG vs GG for COPD vs resistant, Odds ratio (OR) =1.57, 95% confidence limits 0.9-2.7, χ^2 (Yates corrected)= 2.52, p=0.11,

   AA/AG =susceptible (GG=protective)

2. Allele. A vs G for COPD vs resistant, Odds ratio (OR) =1.61, 95% confidence limits 1. 0-2.7, χ^2 (Yates corrected)= 3.38, p=0.07,

   A =susceptible

Table 11b. Tissue Necrosis Factor α -308 G/A polymorphism allele and genotype frequency in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=242 (%)	90 (19%)	394 (81%)	6 (2%)	78 (32%)	158 (65%)
Resistant n=190 (%)	58 (15%)	322 (85%)	3 (2%)	52 (27%)	135 (71%)

* number of chromosomes (2n)

1. Genotype. GG vs AG/AA for COPD vs resistant, Odds ratio (OR) =0.77, 95% confidence limits 0.5-1.2, χ^2 (Yates uncorrected)= 1.62, p=0.20,

   GG=protective (AA/AG =susceptible) trend

2. Allele. A vs G for COPD vs resistant, Odds ratio (OR) =1.3, 95% confidence limits 0.9-1.9, χ^2 (Yates uncorrected)= 1.7, p=0.20,

   A =susceptible trend

Table 12. SMAD3 C89Y polymorphism allele and genotype frequency in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=250 (%)	2 (1%)	498 (99%)	0 (0%)	2 (1%)	248 (99%)
Resistant n=196 (%)	6 (2%)	386 (98%)	0 (0%)	6 (3%)	190 (97%)

* number of chromosomes (2n)

1. Genotype. AA/AG vs GG for COPD vs resistant, Odds ratio (OR) =0.26, 95% confidence limits 0.04-1.4, χ^2 (Yates uncorrected)= 3.19, p=0.07,

   AA/AG =protective (GG susceptible)

Table 13. Intracellular Adhesion molecule 1 (ICAM1) A/G E469K (rs5498) polymorphism allele and genotype frequency in COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=242 (%)	259 (54%)	225 (46%)	73 (30%)	113 (47%)	56 (23%)
Resistant n=182 (%)	217 (60%)	147 (40%)	64 (35%)	89 (49%)	29 (16%)

* number of chromosomes (2n)

1. Genotype. GG vs AG/GG for COPD vs resistant, Odds ratio (OR) =1.60, 95% confidence limits 0.9-2.7, χ^2 (Yates corrected)= 3.37, p=0.07,

   GG =susceptibility

2. Allele. G vs A for COPD vs resistant, Odds ratio (OR) =1.3, 95% confidence limits 1.0-1.7, χ^2 (Yates corrected)= 2.90, p=0.09

Table 14. Caspase (NOD2) Gly881Arg polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	G	C	GG	GC	CC
COPD n=247	486 (98%)	8 (2%)	239 (97%)	8 (3%)	0 (0%)
Resistant n=195 (%)	388 (99.5%)	2 0.5%)	193 (99%)	2 (1%)	0 (0%)

* number of chromosomes (2n)

1. Genotype. CC/CG vs GG for COPD vs resistant, Odds ratio (OR) =3.2, 95% confidence limits 0.6-22, χ^2 (Yates uncorrected)= 2.41, p=0.11 (1-tailed),

   GC/CC=susceptibility (trend)

Table 15. Mannose binding lectin 2(MBL2) +161 G/A polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=218 (%)	110 (25%)	326 (75%)	6 (3%)	98 (45%)	114 (52%)
Resistant n=183 (%)	66 (18%)	300 (82%)	6 (3%)	54 (30%)	123 (67%)

* number of chromosomes (2n)

1. Genotype. GG vs rest for COPD vs resistant, Odds ratio (OR) =0.53, 95% confidence limits 0.4-0.80, χ^2 (Yates uncorrected)= 8.55, p=0.003,

   GG =protective

Table 16. Chymase 1 (CMA1) -1903 G/A promoter polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=239 (%)	259 (54%)	219 (46%)	67 (28%)	125 (52%)	47 (20%)
Resistant n=181 (%)	209 (58%)	153 (42%)	63 (35%)	83 (46%)	35 (19%)

* number of chromosomes (2n)

1. Genotype. AA vs AG/GG for COPD vs resistant, Odds ratio (OR) =0.73, 95% confidence limits 0.5-1.1, χ^2 (Yates corrected)= 1.91, p=0.17,

   AA genotype =protective trend

Table 17. N-Acetyltransferase 2 Arg 197 Gln G/A polymorphism allele and genotype frequencies in COPD and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=247 (%)	136 (28%)	358 (72%)	14 (6%)	108 (44%)	125 (50%)
Resistant n=196 (%)	125 (32%)	267 (68%)	21 (11%)	83 (42%)	92 (47%)

* number of chromosomes (2n)

1. Genotype. AA vs AG/GG for COPD vs resistant, Odds ratio (OR) =0.50, 95% confidence limits 0.2-1.0, χ^2 (Yates uncorrected)= 3.82, p=0.05,

   AA genotype = protective

Table 18. Interleukin 1B (IL-1b) -511 A/G polymorphism allele and genotype frequencies in COPD and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=248 (%)	160 (32%)	336 (68%)	31 (13%)	98 (40%)	119 (48%)
Resistant n=195 (%)	142 (36%)	248 (64%)	27 (14%)	88 (45%)	80 (41%)

* number of chromosomes (2n)

1. Genotype. GG vs AA/AG for COPD vs resistant, Odds ratio (OR) =1.3, 95% confidence limits 0.9-2.0, χ^2 (Yates corrected)= 1.86, p=0.17,

   GG genotype = susceptible trend

Table 19a.Microsomal epoxide hydrolase (MEH) Tyr 113 His T/C (exon 3) polymorphism allele and genotype frequency in COPD and resistant smokers.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
COPD n=249 (%)	137 (28%)	361 (72%)	18 (7%)	101 (41%)	130 (52%)
Resistant n=194 (%)	130 (34%)	258 (66%)	19 (10%)	92 (47%)	83 (43%)

* number of chromosomes (2n)

1. Genotype. TT vs CT/CC for COPD vs resistant, Odds ratio (OR) =1.5, 95% confidence limits 1.0-2.2, χ^2 (Yates corrected)= 3.51, p=0.06,

   TT genotype = susceptible

Table 19b. Microsomal epoxide hydrolase (MEH) His 139 Arg A/G (exon 4) polymorphism allele and genotype frequency in COPD and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=238 (%)	372 (78%)	104 (22%)	148 (62%)	76 (32%)	14 (6%)
Resistant n=179 (%)	277 (77%)	81 (23%)	114 (64%)	49 (27%)	16 (9%)

* number of chromosomes (2n)

1. Genotype. GG vs AA/AG for COPD vs resistant, Odds ratio (OR) =0.64, 95% confidence limits 0.3-1.4, χ^2 (Yates uncorrected)= 1.43, p=0.23,

   GG genotype = protective (trend)

Table 20. Lipo-oxygenase -366 G/A polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
COPD n=247 (%)	21 (4%)	473 (96%)	1 (0.5%)	19 (7.5%)	227 (92%)
Resistant n=192 (%)	25 (7%)	359 (93%)	0 (0%)	25 (13%)	167 (87%)

* number of chromosomes (2n)

1. Genotype. AA/AG vs GG for COPD vs resistant, Odds ratio (OR) =0.60, 95% confidence limits 0.3-1.1, χ^2 (Yates corrected)= 2.34, p=0.12,

   AA/AG genotype = protective (GG susceptible) trend

Table 21. Heat Shock Protein 70 (HSP 70) HOM T2437C polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
COPD n=199 (%)	127 (32%)	271 (68%)	5 (3%)	117 (59%)	77 (39%)
Resistant n=166 (%)	78 (23%)	254 (77%)	4 (2%)	70 (42%)	92 (56%)

* number of chromosomes (2n)

1. Genotype. CC/CT vs TT for COPD vs resistant, Odds ratio (OR) =2.0, 95% confidence limits 1.3-3.1, χ^2 (Yates uncorrected)= 9.52, p=0.002,

   CC/CT genotype = susceptible (TT=protective)

Table 22. Chloride Channel Calcium-activated 1 (CLCA1) +13924 T/A polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	A	T	AA	AT	TT
COPD n=224 (%)	282 (63%)	166 (37%)	84 (38%)	114 (51%)	26 (12%)
Resistant n=158 (%)	178 (56%)	138 (44%)	42 (27%)	94 (59%)	22 (14%)

* number of chromosomes (2n)

1. Genotype. AA vs AT/TT for COPD vs resistant, Odds ratio (OR) =1.7, 95% confidence limits 1.0-2.7, χ^2 (Yates corrected)= 4.51, p=0.03,

   AA=susceptible

Table 23. Monocyte differentiation antigen CD-14 -159 promoter polymorphism allele and genotype frequencies in the COPD patients and resistant smokers.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
COPD n=240 (%)	268 (56%)	212 (44%)	77 (32%)	114 (48%)	49 (20%)
Resistant n=180 (%)	182 (51%)	178 (49%)	46 (25%)	90 (50%)	44 (24%)

* number of chromosomes (2n)

1. Genotype.CC vs CT/TT for COPD vs Resistant, Odds ratio (OR) =1.4, 95% confidence limits 0.9-2.2, χ^2 (Yates uncorrected)= 2.12, p=0.15,

   CC = susceptible (trend)

Table 24. Elafin +49 C/T polymorphism allele and genotype frequencies in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
COPD n=144 (%)	247 (86%)	41 (14%)	105 (73%)	37 (26%)	2 (1%)
Resistant n=75 (%)	121 (81%)	29 (19%)	49 (65%)	23 (31%)	3 (4%)

* number of chromosomes (2n)

1. Genotype. CT/TT vs CC for COPD vs resistant, Odds ratio (OR) = 0.70, 95% confidence limits= 0.4-1.3 , χ^2 (Yates uncorrected)= 1.36, p=0.24,

   CT/TT genotype = protective (trend only)

2. Allele: T vs C for COPD vs resistant, Odds ratio (OR) = 0.69, 95% confidence limits= 0.4-1.2 , χ^2 (Yates uncorrected)= 1.91, p=0.17,

   T genotype = protective (trend only)

Table 25. Beta2-adrenoreceptor Gln 27 Glu polymorphism allele and genotype frequency in the COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	G	CC	CG	GG
Controls n=185 (%)	204 (55%)	168 (45%)	57 (31%)	89 (48%)	39 (21%)
COPD n=238 (%)	268 (56%)	208 (44%)	67 (28%)	134 (56%)	37 (16%)
Resistant n=195 (%)	220 (56%)	170 (44%)	64 (33%)	92 (47%)	39 (20%)

* number of chromosomes (2n)

1. Genotype. GG vs CG/CC for COPD vs resistant, Odds ratio (OR) = 0.74, 95% confidence limits = 0.4-1.2, χ^2 (Yates uncorrected)= 1.47 , p=0.23,

   GG =protective (trend)

2. Genotype. GG vs CG/CC for COPD vs controls, Odds ratio (OR) = 0.69, 95% confidence limits = 0.4-1.2, χ^2 (Yates uncorrected)= 2.16 , p=0.14,

   GG =protective (trend)

Table 26. Maxtrix metalloproteinase 1 (MMP1) -1607 1G/2G polymorphism allele and genotype frequencies in COPD patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	1G	2G	1G1G	1G2G	2G2G
Controls n=174 (%)	214 (61%)	134 (39%)	68 (39%)	78 (45%)	28 (16%)
COPD n=217 (%)	182 (42%)	252 (58%)	47 (22%)	88 (41%)	82 (38%)
Resistant n=187 (%)	186 (50%)	188 (50%)	46 (25%)	94 (50%)	47 (25%)

* number of chromosomes (2n)

1. Genotype. 1G1G vs rest for COPD vs controls, Odds ratio (OR) =0.43, 95% confidence limits 0.3-0.7, χ^2 (Yates uncorrected)= 13.3, p=0.0003

   1G1G genotype =protective
2. Allele. 1G vs 2G for COPD vs controls, Odds ration (OR) =0.45, 95% confidence limits 0.3-0.6, $\chi 2$ (Yates corrected)= 28.8, p<0.0001,

   1G = protective
3. Genotype. 1G1G/1G2G vs rest for COPD vs resistant smokers, Odds ratio (OR) =0.55, 95% confidence limits 0.4-0.9, χ^2 (Yates uncorrected)= 6.83, p=0.009

   1G1G/162G genotypes =protective
4. Allele. 1G vs 2G for COPD vs resistant smokers, Odds ratio (OR) =0.73, 95% confidence limits 0.6-1.0, $\chi 2$ (Yates corrected)= 4.61, p=0.03,

   1G = protective
5. Genotype. 2G2G vs 1G1G/1G2G for COPD vs controls, Odds ratio (OR) =3.17, 95% confidence limits 1.9-5.3, $\chi 2$ (Yates uncorrected)= 21.4, p<0.0001

   2G2G genotype =susceptible
6. Allele. 2G vs 1G for COPD vs controls, Odds ratio (OR) =2.2, 95% confidence limits 1.6-3.0, $\chi 2$ (Yates corrected)= 28.8, p<0.00001,

   2G = susceptible
7. Genotype. 2G2G vs 1G1G/1G2G for COPD vs resistant, Odds ratio (OR) =1.81, 95% confidence limits 1.2-2.9, $\chi 2$ (Yates uncorrected)= 6.83, p=0.009

   2G2G genotype =susceptible
8. Allele. 2G vs 1G for COPD vs resistant, Odds ratio (OR) =1.4, 95% confidence limits 1.0-1.8, $\chi 2$ (Yates corrected)= 4.61, p=0.0.03,

   2G = susceptible

Table 27. Summary table of protective and susceptibility polymorphisms

Gene	Polymorphism	Role
Cyclo-oxygenase 2 (COX2)	COX2 -765 G/C	CC/CG protective
β2-adrenoreceptor (ADBR)	ADBR Arg16Gly	GG susceptible
Interleukin -18 (IL18)	IL18 -133 C/G	CC susceptible
Interleukin -18 (IL18)	IL18 105 A/C	AA susceptible
Plasminogen activator inhibitor 1 (PAI-1)	PAI-1 -675 4G/5G	5G5G susceptible
Nitric Oxide synthase 3 (NOS3)	NOS3 298 Asp/Glu	TT protective
Vitamin D Binding Protein (VDBP)	VDBP Lys 420 Thr	AA/AC protective
Vitamin D Binding Protein (VDBP)	VDBP Glu 416 Asp	TT/TG protective
Glutathione S Transferase (GSTP-1)	GSTP1 Ile105Val	AA protective
Interferon γ (IFN-γ)	IFN-γ 874 A/T	AA susceptible
Interleukin-13 (IL13)	IL13 Arg 130 Gln	AA protective
Interleukin-13 (IL13)	Il13 -1055C/T	TT susceptible
α1-antitrypsin (α1-AT)	α1-AT S allele	MS protective
Tissue Necrosis Factor α TNFα	TNFα +489 G/A	AA/AG susceptible GG protective
Tissue Necrosis Factor α TNFα	TNFα -308 G/A	GG protective AA/AG susceptible
SMAD3	SMAD3 C89Y AG	AA/AG protective GG susceptible
Intracellular adhesion molecule 1 (ICAM1)	ICAM1 E469K A/G	GG susceptible
Caspase (NOD2)	NOD2 Gly 881Arg G/C	GC/CC susceptible
Mannose binding lectin 2 (MBL2)	MBL2 161 G/A	GG protective
Chymase 1 (CMA1)	CMA1 -1903 G/A	AA protective
N- Acetyl transferase 2 (NAT2)	NAT2 Arg 197 Gln G/A	AA protective
Interleukin 1B (IL1B)	(IL1B) -511 A/G	GG susceptible
Microsomal epoxide hydrolase (MEH)	MEH Tyr 113 His T/C	TT susceptible
Microsomal epoxide hydrolase (MEH)	MEH His 139 Arg G/A	GG protective
5 Lipo-oxygenase (ALOX5)	ALOX5 -366 G/A	AA/AG protective GG susceptible
Heat Shock Protein 70 (HSP 70)	HSP 70 HOM T2437C	CC/CT susceptible TT protective
Chloride Channel Calcium-activated 1 (CLCA1)	CLCA1 +13924 T/A	AA susceptible
Monocyte differentiation antigen CD-14	CD-14 -159 C/T	CC susceptible
Elafin	Elafin Exon 1 +49 C/T	CT/TT protective

B2-adrenergic receptor (ADBR)	ADBR Gln 27 Glu C/G	GG protective
Matrix metalloproteinase 1 (MMP1)	MMP1 -1607 1G/2G	1G1G/1G2G protective

Table 28. Combined frequencies of the presence or absence of selected protective genotypes (COX2 (-765) CC/CG, β2 adreno-receptor AA, Interleukin-13 AA, Nitic Oxide Synthase 3 TT and Vitamin D Binding Protein AA) in the smoking subjects (COPD subjects and resistant smokers).

	Number of protective polymorphisms			
Cohorts	0	1	≥2	Total
COPD	136 (54%)	100 (40%)	16 (7%)	252
Resistant smokers	79 (40%)	83 (42%)	34 (17%)	196
% of smokers with COPD	136/215 (63%)	100/183 (55%)	16/50 (32%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, Resist vs COPD	-	-	16.43	0.0003
2+ vs 0-1, Resist vs COPD	3.1	1.6-6.1	12.36	0.0004
1+ vs 0, Resist vs COPD	1.74	1.2-2.6	7.71	0.006

Table 29. Combined frequencies of the presence or absence of selected susceptibility genotypes (Interleukin-18 105 AA, PAI-1 -675 5G5G, Interleukin-13 -1055 TT and Interferon-γ -874 TT genotypes) in the smoking subjects (COPD subjects and resistant smokers).

	Number of susceptibility polymorphisms			
Cohorts	0	1	≥2	Total
COPD	66 (26%)	113 (45%)	73 (29%)	252
Resistant smokers	69 (35%)	92 (47%)	35 (18%)	196
% of smokers with COPD	66/135 (49%)	113/205 (55%)	73/108 (68%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, COPD vs Resist	-	-	8.72	0.01
2+ vs 0-1, COPD vs Resist	1.9	1.2-3.0	6.84	0.009
1+ vs 0, COPD vs Resist	1.5	1.0-3.5	3.84	0.05

Table 30. Combined frequencies of the presence or absence of selected protective genotypes (COX2 (-765) CC/CG, Interleukin-13 AA, Nitic Oxide Synthase 3 TT, Vitamin D Binding Protein AA/AC, GSTP1 AA and α1-antitrypsin MS/SS) in the smoking subjects (COPD subjects and resistant smokers).

	Number of protective polymorphisms			
Cohorts	0	1	≥2	Total
COPD	51 (19%)	64 (24%)	150 (57%)	265
Resistant smokers	16 (8%)	56 (27%)	133 (65%)	205
% of smokers with COPD	51/76 (76%)	64/120 (53%)	150/283 (53%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, Resist vs COPD	-	-	12.14	0.0005
1+ vs 0, Resist vs COPD	2.82	1.5-5.3	11.46	0.0004

Discussion

The above results show that several polymorphisms were associated with either susceptibility and/or resistance to obstructive lung disease in those exposed to smoking environments. The associations of individual polymorphisms on their own, while of discriminatory value, are unlikely to offer an acceptable prediction of disease. However, in combination these polymorphisms distinguish susceptible smokers (with COPD) from those who are resistant. The polymorphisms represent both promoter polymorphisms, thought to modify gene expression and hence protein synthesis, and exonic polymorphisms known to alter amino-acid sequence (and likely expression and/or function) in processes known to underlie lung remodelling. The polymorphisms identified here are found in genes encoding proteins central to these processes which include inflammation, matrix remodelling and oxidant stress.

In the comparison of smokers with COPD and matched smokers with near normal lung function, several polymorphisms were identified as being found in significantly greater or lesser frequency than in the comparator groups (including the blood donor cohort).

- In the analysis of the -765 C/G promoter polymorphisms of cyclo-oxygenase 2 gene, the C allele and CC/CG genotype were found to be significantly greater in the resistant smoker cohort compared to the COPD cohort (OR=1.92, P<0.001

and OR=1.98, P=0.003) consistent with a protective role. The greater frequency compared to the blood donor cohort also suggests that the C allele (CC genotype) is over represented in the resistant group (see Table 1).

- In the analysis of the Arg16Gly polymorphism of the β2 adrenergic receptor gene, the GG genotype was found to be significantly greater in the COPD cohort compared to the controls (OR=1.83, P=0.004) suggesting a possible susceptibility to smoking associated with this genotype. Although the GG genotype is also over-represented in the resistant cohort its effects can be overshadowed by protective polymorphisms (see Table 2).
- In the analysis of the 105 C/A polymorphism of the IL18 gene, the A allele and AA genotype were found to be significantly greater in the COPD cohort compared to the controls (OR=1.46, P=0.02 and OR=1.50, P=0.04 respectively) consistent with a susceptibility role. The AA genotype was also greater in the COPD cohort compared with resistant smokers (OR 1.4, P=0.12) a trend consistent with a susceptibility role (see Table 3a).
- In the analysis of the -133 G/C promoter polymorphism of the IL18 gene, the C allele and CC genotype were found to be significantly greater in the COPD cohort compared to the controls (OR=1.36, P=0.05 and OR=1.44, P=0.06 respectively) consistent with a susceptibility role. The CC genotype was also greater in the COPD cohort compared with resistant smokers a trend consistent with a susceptibility role (see Table 3b).
- In the analysis of the -675 4G/5G promoter polymorphism of the plasminogen activator inhibitor gene, the 5G allele and 5G5G genotype were found to be significantly greater in the COPD cohort compared to the resistant smoker cohort (OR=1.33, P=0.05 and OR=1.55, P=0.08) consistent with a susceptibility role. The greater frequency of the 5G5G in COPD compared to the blood donor cohort also suggests that the 5G5G genotype is associated with susceptibility (see Table 4).
- In the analysis of the 298 Asp/Glu (T/G) polymorphism of the nitric oxide synthase (NOS3) gene, the TT genotype was found to be significantly greater in the resistant smoker cohort compared to the blood donor cohort (OR=2.2, P=0.03) consistent with a protective role. (see Table 5).

- In the analysis of the Lys 420 Thr (A/C) polymorphism of the Vitamin D binding protein gene, the A allele and AA/AC genotype were found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=1.34, P=0.05 and OR=1.39, P=0.10 respectively) consistent with a protective role. (see Table 6a).
- In the analysis of the Glu 416 Asp (T/G) polymorphism of the Vitamin D binding protein gene, the T allele and TT/TG genotype were found to be greater in the resistant smoker cohort compared to the blood donor cohort cohort (OR=1.27, P=0.10 and OR=1.53, P=0.06 respectively) consistent with a protective role. (see Table 6b).
- In the analysis of the Ile 105 Val (A/G) polymorphism of the glutathione S transferase P gene, the A allele and AA genotype were found to be greater in the resistant smoker cohort compared to the blood donor cohort (OR=1.34, P=0.05 and OR=1.45, P=0.07 respectively) consistent with a protective role. (see Table 7).
- In the analysis of the 874 A/T polymorphism of the interferon-γ gene, the AA genotype was found to be significantly greater in the COPD cohort compared to the controls (OR-1.5, P=0.08) consistent with a susceptibility role. (see Table8).
- In the analysis of the Arg 130 Gln (G/A) polymorphism of the Interleukin 13 gene, the AA genotype was found to be greater in the resistant smoker cohort compared to the blood donor cohort (OR=2.94, P=0.09) consistent with a protective role. (see Table 9a).
- In the analysis of the -1055 (C/T) polymorphism of the Interleukin 13 gene, the TT genotype was found to be greater in the COPD cohort compared to the resistant cohort (OR=6.03, P=0.03) consistent with a susceptibility role. (see Table 9b).
- In the analysis of the α1-antitrypsin S polymorphism, the S allele and MS/SS genotype was found to be greater in the resistant smokers compared to COPD cohort (OR=2.42, P=0.01) consistent with a protective role (Table 10).
- In the analysis of the +489 G/A polymorphism of the Tissue Necrosis Factor α gene, the A allele and the AA and AG genotypes were found to be greater in the COPD cohort compared to the controls (OR=1.57, P=0.11) consistent with a

susceptibility role (see Table 11a). Conversely, the GG genotype was found to be greater in the resistant smoker cohort, consistent with a protective role (see Table 11a).

- In the analysis of the -308 G/A polymorphism of the Tissue Necrosis Factor α gene, the GG genotype was found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.77, P=0.20) consistent with a protective role. (see Table 11b). Conversely, the A allele and the AA and AG genotypes were found to be greater in the COPD cohort (OR=1.3, P=0.20), consistent with a susceptibility role (see Table 11b).
- In the analysis of the C89Y A/G polymorphism of the SMAD3 gene, the AA and AG genotypes were found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.26, P=0.07) consistent with a protective role. (see Table 12). Conversely, the GG genotype was found to be greater in the COPD cohort, consistent with a susceptibility role (see Table 12).
- In the analysis of the E469K A/G polymorphism of the Intracellular adhesion molecule 1 gene, the G allele and the GG genotype were found to be greater in the COPD cohort compared to the controls (OR=1.3, P=0.09 and OR=1.6, P=0.07, respectively) consistent with a susceptibility role (see Table 13).
- In the analysis of the Gly 881Arg G/C polymorphism of the Caspase (NOD2) gene, the CC and CG genotypes were found to be greater in the COPD cohort compared to the controls (OR=3.2, P=0.11) consistent with a susceptibility role (see Table 14).
- In the analysis of the 161 G/A polymorphism of the Mannose binding lectin 2 gene, the GG genotype was found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.53, P=0.003) consistent with a protective role. (see Table 15).
- In the analysis of the -1903 G/A polymorphism of the Chymase 1 gene, the AA genotype was found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.73, P=0.17) consistent with a protective role. (see Table 16).
- In the analysis of the Arg 197 Gln G/A polymorphism of the N-Acetyl transferase 2 gene, the AA genotype was found to be greater in the resistant

smoker cohort compared to the COPD cohort (OR=0.50, P=0.05) consistent with a protective role. (see Table 17).

- In the analysis of the -511 A/G polymorphism of the Interleukin 1B gene, the GG genotype was found to be greater in the COPD cohort compared to the controls (OR=1.3, P=0.17) consistent with a susceptibility role (see Table 18).
- In the analysis of the Tyr 113 His T/C polymorphism of the Microsomal epoxide hydrolase gene, the TT genotype was found to be greater in the COPD cohort compared to the controls (OR=1.5, P=0.06) consistent with a susceptibility role (see Table 19a).
- In the analysis of the Arg 139 G/A polymorphism of the Microsomal epoxide hydrolase gene, the GG genotype was found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.64, P=0.23) consistent with a protective role. (see Table 19b).
- In the analysis of the -366 G/A polymorphism of the 5 Lipo-oxygenase gene, the AG and AA genotypes were found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.60, P=0.12) consistent with a protective role. (see Table 20). Conversely, the GG genotype was found to be greater in the COPD cohort, consistent with a susceptibility role (see Table 20).
- In the analysis of the HOM T2437C polymorphism of the Heat Shock Protein 70 gene, the CC and CT genotypes were found to be greater in the COPD cohort compared to the controls (OR=2.0, P=0.002) consistent with a susceptibility role (see Table 21). Conversely, the TT genotype was found to be greater in the resistant smoker cohort, consistent with a protective role (see Table 21).
- In the analysis of the +13924 T/A polymorphism of the Chloride Channel Calcium-activated 1 gene, the AA genotype was found to be greater in the COPD cohort compared to the controls (OR=1.7, P=0.03) consistent with a susceptibility role (see Table 22).
- In the analysis of the -159 C/T polymorphism of the Monocyte differentiation antigen CD-14 gene, the CC genotype was found to be greater in the COPD cohort compared to the controls (OR=1.4, P=0.15) consistent with a susceptibility role (see Table 23).

- In the analysis of the Exon 1 +49 C/T polymorphism of the Elafin gene, the T allele and the CT and TT genotypes were found to be greater in the resistant smoker cohort compared to the COPD cohort (OR=0.69, P= 0.17, OR=0.70, P=0.24, respectively) consistent with a protective role. (see Table 24).
- In the analysis of the Gln 27 Glu C/G polymorphism of the β2-adrenergic receptor gene, the GG genotype was found to be greater in the resistant smoker cohort and the blood donor controls compared to the COPD cohort (OR=0.74, P=0.23, OR=0.69, P=0.14, respectively) consistent with a protective role. (see Table 25).
- In the analysis of the -1607 1G/2G promoter polymorphism of the MMP1 gene, the 1G allele and 1G1G/1G2G genotypes were found to be significantly greater in the resistant smoker cohort compared to the COPD cohort (OR=0.73, p=0.03 and OR=0.55, p=0.009), consistent with a protective role. The greater frequency of the 1G1G in the resistant group compared to the blood donor cohort also suggests that the 1G allele is protective (see Table 26).

It is accepted that the disposition to chronic obstructive lung diseases (eg. emphysema and COPD) is the result of the combined effects of the individual's genetic makeup and their lifetime exposure to various aero-pollutants of which smoking is the most common. Similarly it is accepted that COPD encompasses several obstructive lung diseases and characterised by impaired expiratory flow rates (eg FEV1). The data herein suggest that several genes can contribute to the development of COPD. A number of genetic mutations working in combination either promoting or protecting the lungs from damage can be involved in elevated resistance or susceptibility.

From the analyses of the individual polymorphisms, 19 protective genotypes were identified and analysed for their frequencies in the smoker cohort consisting of resistant smokers and those with COPD. When the frequencies of resistant smokers and smokers with COPD were compared according to the presence of 0, 1 and 2+ protective genotypes (out of COX2 CC/CG, β2 adreno-receptor Arg 16 Gly AA, Interleukin-13 Arg 130 Gln AA, Nitic Oxide Synthase 3 298 TT and Vitamin D Binding Protein 420 AA/AC) significant differences were found (overall $\chi 2$=16.43, P=0.0003) suggesting that smokers with 2+ protective genotypes had three times more likelihood of being resistant (OR=3.1, P=0.004) while those no protective genotypes were nearly twice as

likely to have COPD (OR=1.74, P=0.006) (see Table 28). Examined another way, the chances of having COPD diminished from 63%, 55% to 32% in smokers with 0, 1 and 2+ of the protective genotypes tested for respectively. On analysis of a selection of the protective genotypes (out of COX2 CC/CG, NOS3 298 TT, VDBP-420 AA/AC, VDBP-416 TT/TG, GSTP1 AA, IL-13-140 AA, and α1-AT MS/SS), a significant difference in frequency of COPD versus resistance was found in those with 0 versus 1+ of the protective genotypes tested for (OR=2.82, P=0.0004)(see Table 30), showing a 2-3 fold increase in COPD in those with 0 of the protective genotypes tested for .

From the analyses of the individual polymorphisms, 17 susceptibility genotypes were identified and analysed for their frequencies in the smoker cohort consisting of resistant smokers and those with COPD. When the frequencies of resistant smokers and smokers with COPD were compared according to the presence of 0, 1 and 2+ susceptibility genotypes (out of Interleukin-18 105 AA, PAI-1 -675 5G5G, Interleukin-13 -1055 TT and Interferon-γ -874 TT genotypes) significant differences were found (overall $\chi 2$=8.72, P=0.01) suggesting that smokers with 2+ of the susceptibility genotypes tested for had two times more likelihood of having COPD (OR=1.9, P=0.009) while those with none of the susceptibility genotypes tested for were 1.5 fold as likely to have COPD (OR=1.5, P=0.05) (see Table 29). Examined another way, the chances of having COPD increased from 49%, 55% to 68% in smokers with 0, 1 and 2+ of the susceptibility genotypes tested for respectively.

These findings indicate that the methods of the present invention can be predictive of COPD, emphysema, or both COPD and emphysema in an individual well before symptoms present.

These findings therefore also present opportunities for therapeutic interventions and/or treatment regimens, as discussed herein. Briefly, such interventions or regimens can include the provision to the subject of motivation to implement a lifestyle change, or therapeutic methods directed at normalising aberrant gene expression or gene product function. For example, the -765 G allele in the promoter of the gene encoding COX2 is associated with increased expression of the gene relative to that observed with the C allele. As shown herein, the C allele is protective with respect to predisposition to or potential risk of developing COPD, emphysema, or both COPD and emphysema, whereby a suitable therapy in subjects known to possess the -765 G allele can be the administration of an agent capable of reducing expression of the gene encoding COX2.

An alternative suitable therapy can be the administration to such a subject of a COX2 inhibitor such as additional therapeutic approaches, gene therapy, RNAi. In another example, as shown herein the -133 C allele in the promoter of the gene encoding IL18 is associated with susceptibility to COPD, emphysema, or both COPD and emphysema. The -133 G allele in the promoter of the gene encoding IL18 is associated with increased IL18 levels, whereby a suitable therapy in subjects known to possess the -133 C allele can be the administration of an agent capable of increasing expression of the gene encoding IL18. In still another example, as shown herein the -675 5G5G genotype in the promoter of the plasminogen activator inhibitor gene is associated with susceptibility to COPD, emphysema, or both COPD and emphysema. The 5G allele is reportedly associated with increased binding of a repressor protein and decreased transcription of the gene. A suitable therapy can be the administration of an agent capable of decreasing the level of repressor and/or preventing binding of the repressor, thereby alleviating its downregulatory effect on transcription. An alternative therapy can include gene therapy, for example the introduction of at least one additional copy of the plasminogen activator inhibitor gene having a reduced affinity for repressor binding (for example, a gene copy having a -675 4G4G genotype).

Suitable methods and agents for use in such therapy are well known in the art, and are discussed herein.

The identification of both susceptibility and protective polymorphisms as described herein also provides the opportunity to screen candidate compounds to assess their efficacy in methods of prophylactic and/or therapeutic treatment. Such screening methods involve identifying which of a range of candidate compounds have the ability to reverse or counteract a genotypic or phenotypic effect of a susceptibility polymorphism, or the ability to mimic or replicate a genotypic or phenotypic effect of a protective polymorphism.

Still further, methods for assessing the likely responsiveness of a subject to an available prophylactic or therapeutic approach are provided. Such methods have particular application where the available treatment approach involves restoring the physiologically active concentration of a product of an expressed gene from either an excess or deficit to be within a range which is normal for the age and sex of the subject. In such cases, the method comprises the detection of the presence or absence of a susceptibility polymorphism which when present either upregulates or downregulates

expression of the gene such that a state of such excess or deficit is the outcome, with those subjects in which the polymorphism is present being likely responders to treatment.

Table 31 below presents representative examples of polymorphisms in linkage disequilibrium with the polymorphisms specified herein. Examples of such polymorphisms can be located using public databases, such as that available at www.hapmap.org. Specified polymorphisms are indicated in the columns marked SNP NAME. Unique identifiers are indicated in the columns marked RS NUMBER.

Table 31. Polymorphisms reported to be in linkage disequilibrium (unless stated) with the specified polymorphism.

SNP NAME	RS NUMBER	SNP NAME	RS NUMBER	SNP NAME	RS NUMBER
	COX2 SNPs		rs6684912		rs5277
	rs7527769		rs2745559		rs2066823
	rs7550380		rs12042763		rs4648263
	rs2206594		rs4648250		rs4987012
	rs6687495		rs4648251		rs20428
	rs6681231		rs2223626		rs20429
	rs13376484		rs689462		rs4648264
	rs12064238		rs4648253		rs4648265
	rs10911911		rs689465		rs4648266
	rs12743673		rs12027712		rs4648267
	rs10911910		rs689466		rs11567824
	rs12743516		rs2745558		rs4648268
	rs10911909		rs3918304		rs4648269
	rs1119066		rs20415		rs4648270
	rs1119065		rs20416		rs12759220
	rs1119064		rs4648254		rs20430
	rs10798053		rs11567815		rs4648271
	rs12409744	-765G>C	rs20417		rs11567825
	rs10911908		rs4648256		rs4648273
	rs10911907		rs20419		rs16825748
	rs7416022		rs2734779		rs4648274
	rs2745561		rs20420		rs16825745
	rs10911906		rs20422		rs20432
	rs2734776		rs20423		rs20433
	rs2734777		rs5270		rs3218622
	rs12084433		rs20424		rs2066826
	rs2734778		rs5271		rs5278
	rs2745560		rs4648257		rs4648276
	rs2223627		rs11567819		rs20434
	rs2383517		rs3134591		rs3218623
	rs4295848		rs3134592		rs3218624
	rs4428839		rs20426		rs5279
	rs4609389		rs4648258		rs4648278
	rs4428838		rs11567820		rs13306034

rs12131210
rs2179555
rs2143417
rs2143416
rs11583191
rs2383516
rs2383515
rs10911905
rs10911904

rs2745557
rs11567821
rs4648259
rs4648260
rs4648261
rs4648262
rs11567822
rs11567823
rs2066824
rs20427

rs2853803
rs4648279
rs4648281
rs4648282
rs11567826
rs4648283
rs4648284
rs4648285
rs11567827
rs4648286

rs4648287
rs5272
rs4648288
rs5273
rs5274
rs3218625
rs4648289
rs4648290
rs1051896
rs5275
1ADRB SNPs
rs2082382
rs2082394
rs2082395
rs9325119
rs9325120
rs12189018
rs11168066
rs11959615
rs11958940
rs4705270
rs10079142
rs9325121
rs11746634
rs11168067
rs9325122
rs11957351
rs11948371
rs11960649
rs1432622
rs1432623
rs11168068
rs17778257
rs2400706
rs2895795
rs2400707
rs2053044
rs17108803
rs12654778
rs11168070
rs11959427

rs1042719
rs3729944
rs3730182
rs1042720
rs6879202
rs3777124
rs1803051
rs8192451
rs4987255
rs3177007
rs1126871
rs6885272
rs6889528
rs4521458
rs10463409
rs7702861
IL-18 SNPs
rs187238
rs5744228
rs360718
rs360717
rs5744229
rs100000353
rs5744231
rs5744232
rs7106524
rs189667
rs12290658
rs12271175
rs11606049
rs360716
rs360715
rs360714
rs2043055
rs5744233
rs795467
rs12270240
rs100000354
rs4937113
rs100000355
rs360723

−133 C/G

105 A/C

-675 4G/5G

rs5744244
rs360722
rs5023207
rs5744246
rs5744247
rs360721
rs4988359
rs12721559
rs5744248
rs5744249
rs5744250
rs5744251
rs100000356
rs1834481
rs17215057
rs5744253
rs5744254
rs5744255
rs5744256
rs5744257
rs360720
rs5744258
rs5744259
rs5744260
rs5744261
rs549908
PAI-1 SNPs
rs6465787
rs7788533
rs6975620
rs6956010
rs12534508
rs4729664
rs2527316
rs2854235
rs10228765
rs2854225
rs2854226
rs2227707
rs2227631
No rs

	rs1042711	rs5744237	**NOS3 SNPs**
	rs1801704	rs5744238	rs2373962
Arg16Gly	rs1042713	rs5744239	rs2373961
	rs1042714	rs7932965	rs6951150
	rs1042717	rs11214103	rs13238512
	rs1800888	rs5744241	rs10247107
	rs1042718	rs5744242	rs10276930
	rs3729943	rs5744243	rs10277237
	rs12703107	rs9282804	rs2282679
	rs6946340	Asp298Glu	rs1799983	rs2282680
	rs6946091	**VDBP SNPs**	rs705117
	rs6946415	rs222035	rs2070741
	rs10952296	rs222036	rs2070742
	rs13309715	rs16846943	rs6821541
	rs10952297	rs7668653	rs222048
	rs7784943	rs1491720	rs432031
	rs11771443	rs16845007	rs432035
	rs2243310	rs17830803	rs222049
	rs1800783	Glu416Asp	rs7041	rs222050
	rs3918155	Lys420Thr	rs4588	rs12510584
	rs3918156	rs3737553	rs17467825
	rs2566519	rs9016	**GSTP1 SNPs**
	rs3918157	rs1352846	rs656652
	rs3918158	rs222039	rs625978
	rs3918159	rs3775154	rs6591251
	rs2566516	rs222040	rs12278098
	rs3918225	rs843005	rs612020
	rs3918160	rs222041	rs12284337
	rs1800779	rs7672977	rs12574108
	rs2243311	rs705121	rs6591252
	rs3918161	rs11723621	rs597717
	rs10952298	rs2298850	rs688489
	rs2070744	rs705120	rs597297
	rs3918226	rs2298851	rs6591253
	rs3918162	rs844806	rs6591254
	rs3918163	rs1491709	rs7927381
	rs3918164	rs705119	rs7940813
	rs3918165	rs6845925	rs593055
	rs1800781	rs12640255	rs7927657
	rs13310854	rs12644050	rs614080
	rs13310763	rs6845869	rs7941395
	rs2853797	rs12640179	rs7941648
	rs13311166	rs222042	rs7945035
	rs13310774	rs3187319	rs2370141
	rs2853798	rs222043	rs2370142
	rs11974098	rs842999	rs7949394
	rs3918166	rs222044	rs7949587
	rs3730001	rs222045	rs6591255
	rs3918167	rs16846912	rs8191430
	rs3918168	rs222046	rs6591256
	rs3918169	rs705118	rs8191431

	rs3918170		rs222047	rs8191432
	rs3793342		rs13142062	rs7109914
	rs3793341		rs843000	rs4147580
	rs1549758		rs3755967	rs8191436
	rs1007311		rs1491710	rs8191437
	rs9282803		rs2282678	rs17593068
	rs8191438		rs2069718	rs7145047
	rs8191439		rs3087272	rs7141735
	rs8191440		rs2069719	rs11558264
	rs8191441		rs9282708	rs6647
	rs1079719		rs2069720	rs8350
	rs1871041		rs1042274	rs2230075
	rs4147581		rs2069721	rs1049800
	rs8191444		rs2069734	S allele rs17580
	rs8191445		rs2069722	rs2854258
	rs2370143		rs2234687	rs2753937
	rs8191446		rs7957366	rs2749547
	rs3891249		rs2069723	rs1243162
	rs8191447		rs2069724	rs2753938
	rs12796085		rs2069725	rs2070709
	rs8191448		rs4394909	rs17090719
	rs762803		rs2069726	rs11846959
	rs8191449		rs2069727	rs1802962
Ile105Val	rs947894		**IL-13 SNPs**	rs2749521
	rs4986948	-1055 C/T	rs1800925	rs2753939
	rs675554		rs11575055	rs1802959
	rs749174		rs2069755	rs1802961
	rs8191450		rs2069741	rs1050469
	rs743679		rs2069742	Z allele no rs
	rs1799811		rs2069743	rs1050520
	rs11553890		rs2069756	rs12077
	rs4986949		rs3212142	**rs12233**
	rs8191451		rs2066960	**rs13170**
	rs1871042		rs1295687	**rs1303**
	rs11553892		rs3212145	rs1802960
	rs4891		rs2069744	rs1243163
	rs6413486		rs2069745	rs2073333
	rs5031031		rs2069746	rs1243164
	rs947895		rs2069747	rs7144409
	IFN- SNPs		rs2069748	rs7142803
	rs2069707		rs1295686	rs1243165
	rs3814242	Arg130Gln	rs20541	rs1051052
	rs2069709		rs2069749	rs1243166
	rs2069710		rs1295685	rs11628917
	rs2069711		rs848	rs11832
	rs2069712		rs2069750	rs9944155
874 A/T	rs2430561		rs847	1237 G/A rs11568814
	rs2069713		**a1-antitrypsin SNPs**	rs877081
	rs1861494		rs709932	rs877082
	rs2234685		rs11558261	rs877083

	rs1861493	rs20546	rs877084
	rs2069714	rs11558263	rs875989
	rs2069715	F1028580	rs9944117
	rs2069716	rs7145770	rs1884546
	rs2069717	rs2239652	rs1884547
	rs1885065	rs2735442	rs8046608
	rs1884548	rs2569693	rs5743264
	rs1243167	rs281439	rs5743266
	rs17751614	rs281440	rs2076752
	rs1884549	rs2569694	rs5743267
	rs1243168	rs11575073	rs8061316
	rs17090693	rs2569695	rs8061636
	rs17824597	rs2075741	rs16948754
	TNFa SNPs	rs11575074	rs7206340
	rs1799964	rs2569696	rs2076753
	rs1800630	rs2735439	rs2067085
	rs1799724	rs2569697	rs16948755
+489 G/A	rs1800610	rs2075742	rs2111235
	rs3093662	rs2569698	rs2111234
	rs3093664	rs11669397	rs7190413
-308 G/A	rs1800629 (1)	rs901886	rs7206582
	SMAD3 SNPs	rs885742	rs8045009
C89Y	C89Y no rs (2)	rs2569699	rs6500328
	ICAM1	rs1056538	rs7500036
	rs1799969	rs11549918	rs8057341
	rs5493	rs2569700	rs12918060
	rs5030381	rs2228615	rs7204911
	rs5494	rs2569701	rs7500826
	rs3093033	rs2569702	rs4785449
	rs5495	rs2735440	rs12922299
	rs1801714	rs2569703	rs11649521
	rs13306429	rs10418913	rs13339578
	rs2071441	rs1056536	rs17221417
	rs5496	rs2569704	rs13331327
	rs5497	rs11673661	rs11642482
	rs13306430	rs2569705	rs11642646
E469K	rs5498	rs10402760	rs17312836
	rs5030400	rs2569706	rs5743268
	rs2071440	rs2569707	rs5743269
	rs5499	rs2735441	rs5743270
	rs3093032	rs2436545	rs12925051
	rs1057981	rs2436546	rs12929565
	rs5500	rs2916060	rs13380733
	rs5501	rs2916059	rs13380741
	rs5030383	rs2916058	rs11647841
	rs281436	rs2569708	rs10451131
	rs923366	rs12972990	rs2066842
	rs281437	rs735747	rs5743271
	rs3093030	rs885743	rs7498256
	rs5030384	**NOD2 SNPs**	rs5743272
	rs5030385	rs4785224	rs5743273

	rs3810159	rs5743261	rs2076754
	rs281438	rs5743262	rs2066843
	rs3093029	rs5743263	rs1078327
	rs5743274	rs11645386	rs1031101
	rs1861759	rs7187857	rs10824795
	rs5743275	rs8061960	rs10824794
	rs5743276	rs5743294	rs920725
	rs2066844	rs2357791	rs7916582
	rs5743277	rs7359452	rs920724
	rs5743278	rs7203344	rs16933335
	rs6413461	rs5743295	rs11003125
	rs3813758	rs5743296	rs7100749
	rs5743279	rs3135499	rs11003124
	rs5743280	rs5743297	rs7084554
	rs5743281	rs5743298	rs7096206
	rs4785225	rs5743299	rs11003123
	rs16948773	rs3135500	rs11575988
	rs9931711	rs5743300	rs11575989
	rs17313265	rs8056611	rs7095891
	rs11646168	rs2357792	rs4647963
	rs9925315	rs12600253	rs8179079
	rs5743284	rs12598306	rs5030737
	rs5743285	rs7205423	161 G/A rs1800450
	rs751271	rs718226	rs1800451
	rs748855	**MBL2 SNPs**	rs12246310
	rs1861758	rs7899547	rs12255312
	rs13332952	rs10824797	rs11003122
	rs7198979	rs11003131	rs1982267
	rs1861757	rs930506	rs1982266
	rs7203691	rs930505	rs4935047
	rs5743286	rs11003130	rs4935046
	rs5743287	rs2384044	rs10824793
	rs10521209	rs2384045	rs1838066
Gly881Arg	rs2066845	rs5027257	rs1838065
	rs5743289	rs2384046	rs930509
	rs8063130	rs12263867	rs930508
	rs2076756	rs11003129	rs930507
	rs12920425	rs12221393	**CMA1 SNPs**
	rs12920040	rs2165811	rs1956920
	rs12920558	rs12782244	rs1956921
	rs12919099	rs11003128	-1903 G/A rs1800875
	rs12920721	rs17664818	rs1800876
	rs2076755	rs7475766	rs3759635
	rs5743290	rs10824796	rs1956922
	rs5743291	rs16933417	rs1956923
	rs11642651	rs2165810	**NAT2 SNPs**
	rs1861756	rs11003127	rs11780272
	rs749910	rs3925313	rs2101857
	rs4990643	rs7094151	rs13363820
	rs1077861	rs7071882	rs6984200
	rs5743292	rs12264958	rs13277605

	rs9921146		rs11003126	rs9987109
	rs7820330		rs7596849	-366 G/A rs9550373
	rs7460995		rs4848306	rs11542984
	rs2087852		rs3087257	rs4769055
	rs2101684		rs7556811	rs17074937
	rs7011792		rs7556903	rs9671065
	rs1390358		rs6743438	rs9579645
	rs923796		rs6743427	rs9579646
	rs4546703		rs6761336	rs4075131
	rs4634684		rs6761335	rs4075132
	rs2410556		rs6743338	rs9315043
	rs11996129		rs6761245	rs9315044
	rs4621844		rs6761237	rs4597169
	rs11785247		rs6743330	rs9578037
	rs1115783		rs6743326	rs9578196
	rs1115784		rs6743322	rs4293222
	rs1961456		rs6761220	rs10507391
	rs1112005		rs6761218	rs12429692
	rs11782802		rs5021469	rs4769871
	rs973874		rs6710598	rs4769872
	rs1495744		rs1143623	rs4769873
	rs7832071		rs1143624	rs12430051
	rs1805158		rs2708920	rs9315045
	rs1801279		rs1143625	rs9670278
	rs1041983		rs2853545	rs4503649
	rs1801280		rs2708921	rs9508832
	rs4986996		rs1143626	rs9670460
	rs12720065		rs3087258	rs3885907
	rs4986997	C-511T	rs16944	rs3922435
	rs1799929		rs3917346	rs9551957
Arg197Gln	rs1799930		rs4986962	rs12018461
	rs1208		rs1143627	rs9551958
	rs1799931		**MEH SNPs**	rs10467440
	rs2552	Tyr113His	rs1051740 (2)	rs12017304
	rs4646247	His139Arg	rs2234922 (2)	rs9551959
	rs971473		**ALOX5AP SNPs**	rs11617473
	rs721398		rs4076128	rs11147438
	IL-1B SNPs		rs9508830	rs10162089
	rs10169916		rs4073259	rs9551960
	rs13009179		rs4073260	rs9285075
	rs4849127		rs11616333	rs12431114
	rs4849126		rs4073261	rs4254165
	rs7558108		rs4075474	rs4360791
	rs13032029		rs4075473	rs17612031
	rs13013349		rs9670115	rs3803277
	rs12623093		rs9315042	rs3803278
	rs3087255		rs3809376	rs12429469
	rs3087256		rs12877064	rs17612099
	rs6721954		rs9508831	rs9550576
	rs12621220		rs9670503	rs4356336

rs4584668
rs4238137
rs17612127
rs4147063
rs4147064
rs4147062
rs9315046
rs9506352
rs9670531
rs9671182
rs9315047
rs17690694
rs9652070
rs17074966
rs4387455
rs4254166
rs4075692
rs17690748
rs9671124
rs9671125
rs9741436
rs9578197
rs4769056
rs11147439
rs12721459
rs4769874

HSP70 HOM SNPs

rs1043618
rs11576009
rs11557922
rs11576010
rs1008438
rs11576011
rs4713489
rs16867582
rs12526722
rs6933097
rs12213612
rs481825
rs7757853
rs7757496
rs9469057
rs12182397
rs16867580
rs2075799
rs482145
rs2227957
T2437C rs2227956
rs2227955

rs2075800

CLCA1 SNPs

rs2791519
rs2791518
rs5744302
rs1321697
rs2753338
rs2791517
rs5744303
rs2734706
rs2753345
rs2753347
rs2753348
rs2753349
rs5744304
rs5744305
rs1358826
rs2753359
rs5744306
rs2734711
rs5744307
rs2734712
rs2753361
rs2753364
rs1555389
rs2753365
rs100000100
rs100000101
rs5744310
rs5744311
rs5744312
rs4656114
rs5744313
rs2753367
rs4656115
rs2734713
rs5744314
rs5744315
rs5744316
rs5744317
rs5744318
rs926063
rs5744319
rs5744320
rs5744321
rs5744322
rs5744323
rs5744324
rs2791516

rs2734714
rs6661730
rs2753377
rs2753378
rs2145412
rs2180762
rs1005569
rs5744325
rs5744326
rs1985554
rs1985555
rs100000102
rs100000103
rs1969719
rs2390102
rs5744329
rs1407142
rs2753384
rs2753385
rs5744330
rs5744331
rs926064
rs926065
rs926066
rs926067
rs2753386
rs2180764
rs2734689
rs5744332
rs5744333
rs11161837
rs5744335
rs2038485
rs3765989
rs2734690
rs5744336
rs2734691
rs2734692
rs5744337
rs5744338
rs2734694
rs5744339
rs100000104
rs2791515
rs4656116
rs5744342
rs5744343
rs2180761
rs5744344

rs5744345
rs1358825

rs5744443
rs5744444

rs6032038
rs6032039

	rs2145410		rs3138074	rs2267863
	rs2734695		rs13166911	rs6124692
	rs5744346		rs2563310	+49 C/T No rs
	rs5744347		rs2569193	rs17333103
	rs100000105		rs2569192	rs17333180
	rs5744349		rs5744446	rs1983649
	rs4655913		rs5744447	rs16989785
	rs1321696		rs5744448	rs17424356
	rs5744352		rs3138076	rs6017500
	rs11583355		rs12519656	rs6032040
	rs100000106		rs5744449	rs6017501
	rs1321695		rs2915863	rs2664581
+13924 T/A	rs1321694		rs3138078	rs17424474
	rs2791514		rs6875483	rs17333381
	rs2734696		rs2569191	rs1053826
	rs5744354		rs5744451	rs2664533
	rs2791513		rs5744452	rs1053831
	rs2753332		rs100000098	rs2664520
	rs2791512		rs17118968	rs2267864
	rs2791511		rs5744455	rs13038355
	rs2734697	-159 C/T	rs2569190	rs13043296
	CD14 SNPs		rs2569189	rs13039213
	rs6877461		rs2563303	rs6104049
	rs3822356		rs3138079	rs13043503
	rs6877437		rs2228049	rs6104050
	rs12153256		rs13763	rs17424578
	rs11554680		rs11556179	rs17424613
	rs12109040		rs4914	rs6017502
	rs12517200		**Elafin SNPs**	rs6094101
	rs5744430		rs2868237	rs6130778
	rs5744431		rs4632412	rs6130779
	rs100000092		rs7347427	rs6104051
	rs5744433		rs6032032	rs6104052
	rs100000093		rs10854230	**ADBR2 SNPs**
	rs4912717		rs7347426	rs2082382
	rs100000094		rs8183548	rs2082394
	rs100000095		rs6104047	rs2082395
	rs100000096		rs6513967	rs9325119
	rs6864930		rs13038813	rs9325120
	rs100000097		rs8118673	rs12189018
	rs6864583		rs7346463	rs11168066
	rs6864580		rs7362841	rs11959615
	rs6889418		rs13042694	rs11958940
	rs6889416		rs13038342	rs4705270
	rs5744440		rs7363327	rs10079142
	rs5744441		rs6073668	rs9325121
	rs5744442		rs13044826	rs11746634
	rs11168067		rs1800468	rs542603
	rs9325122		rs4987025	rs574939
	rs11957351		rs1800469	rs573764
	rs11948371		rs11466314	rs7102189

	rs11960649		rs12977628	rs575727
	rs1432622		rs12977601	rs552306
	rs1432623		rs12985978	rs634607
	rs11168068		rs11466315	rs12286876
	rs17778257		rs11551223	rs12285331
	rs2400706		rs11551226	rs519806
	rs2895795		rs11466316	rs12283571
	rs2400707		rs13306706	rs2839969
	rs2053044		rs13306707	rs2000609
	rs17108803		rs13306708	rs7125865
	rs12654778		rs9282871	rs570662
	rs11168070	Leu10Pro	rs1982073	rs11225427
	rs11959427		rs1800471	rs484915
	rs1042711		rs13447341	rs470307
	rs1801704		rs11466318	rs2408490
	rs1042713		rs12976890	rs12279710
Gln27Glu	rs1042714		rs12978333	rs685265
	rs1042717		rs10420084	rs7107224
	rs1800888		rs10418010	rs1155764
	rs1042718		rs12983775	rs534191
	SOD3 SNPs		rs12462166	rs509332
Arg213Gly	rs1799895 (2)		rs2241715	rs12283759
	TGFB1 SNPs		rs9749548	rs2105581
	rs1529717		rs7258445	rs470206
	rs1046909		rs11466320	rs533621
	rs2241712		rs11466321	-1607 G/GG rs1799750
	rs2241713		rs8108052	rs470211
	rs2241714		rs6508976	rs470146
	rs11673525		rs8108632	rs2075847
	rs2873369		rs11466324	rs473509
	rs11083617		rs2241716	rs498186
	rs11083616		rs2241717	**GSTM1 polymorphism**
	rs4803458		rs2288873	Null Null allele No rs (2)
	rs11670143		rs12973435	**MMP9 SNPs**
	rs1982072		rs2014015	rs11696804
	rs11668109		rs1989457	rs6104416
	rs13345981		rs10406816	rs3933239
	rs11666933		rs8102918	rs3933240
	rs11466310		rs4803455	rs6094237
	rs11466311		**MMP1 SNPs**	rs11697325
	rs2317130		rs529381	rs6130988
	rs4803457		rs1144396	rs6073983
	rs3087453		rs504875	rs6130989
	rs1800820		rs526215	rs6130990
	rs1054797		rs12280880	rs10211842
	rs6073984		rs8125587	**TIMP3 SNPs**
	rs6073985		rs3918253	rs5754289
	rs8121146		rs2274755	rs5754290
	rs6032620		rs2664538	rs9606994
	rs11698788		rs3918254	rs7285034

	rs6032621		rs6130993	rs13433582
	rs6065912		rs3918255	rs1962223
	rs6104417		rs2236416	rs8137129
	rs3848720		rs6130994	rs1807471
	rs13040272		rs3918256	rs7290885
	rs6104418		rs3918281	rs5749511
	rs3848721		rs3787268	rs11703366
	rs3848722		rs3918257	rs4990774
	rs6104419		rs6017725	-1296 T/C rs9619311
	rs4810482		rs6032623	rs2234921
	rs3761157		rs3918258	rs2234920
	rs3761158		rs2250889	rs16991235
	rs3761159		rs3918259	rs4638893
	rs8113877		rs3918260	rs12169569
	rs6065913		rs13969	rs5998639
	rs6104420		rs6104427	rs7284166
	rs6104421		rs6104428	rs5749512
	rs3918240		rs2274756	
	rs6104422		rs6017726	
	rs3918278		rs3918261	
	rs3918241		rs6032624	
-1562 C/T	rs3918242		rs3918262	
	rs3918243		rs3918263	
	rs3918279		rs3918264	
	rs3918280		rs6130995	
	rs4578914		rs6130996	
	rs6017724		rs3918265	
	rs3918244		rs3918266	
	rs3918245		rs3918267	
	rs6130992		rs6073987	
	rs3918247		rs6073988	
	rs3918248		rs3918282	
	rs3918249		rs1802909	
	rs6104423		rs13925	
	rs6104424		rs20544	
	rs6104425		rs1056628	
	rs6104426		rs1802908	
	rs3918250		rs2664517	
	rs1805089		rs9509	
	rs3918251		rs3918268	
	rs13040572		rs3918269	
	rs13040580		rs3918270	
	rs3918252		**MMP12 SNPs**	
	rs8125581	-82 A/G	rs2276109 (2)	

(1 = no other SNPs reported to be in LD, 2=no other SNPS reported to be in LD)

INDUSTRIAL APPLICATION

The present invention is directed to methods for assessing a subject's risk of developing chronic obstructive pulmonary disease (COPD), emphysema, or both COPD

and emphysema. The methods comprise the analysis of polymorphisms herein shown to be associated with increased or decreased risk of developing COPD, emphysema, or both COPD and emphysema, or the analysis of results obtained from such an analysis. The use of polymorphisms herein shown to be associated with increased or decreased risk of developing COPD, emphysema, or both COPD and emphysema in the assessment of a subject's risk are also provided, as are nucleotide probes and primers, kits, and microarrays suitable for such assessment. Methods of treating subjects having the polymorphisms herein described are also provided. Methods for screening for compounds able to modulate the expression of genes associated with the polymorphisms herein described are also provided.

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All patents, publications, scientific articles, and other documents and materials referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced document and material is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such patents, publications, scientific articles, web sites, electronically available information, and other referenced materials or documents.

The specific methods and compositions described herein are representative of various embodiments or preferred embodiments and are exemplary only and not

intended as limitations on the scope of the invention. Other objects, aspects, examples and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably can be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. Thus, for example, in each instance herein, in embodiments or examples of the present invention, any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms in the specification, thus indicating additional examples, having different scope, of various alternative embodiments of the invention. Also, the terms "comprising", "including", containing", etc. are to be read expansively and without limitation. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. It is also that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those

skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

CLAIMS:

1. A method of determining a subject's risk of developing one or more obstructive lung diseases comprising analysing a sample from said subject for the presence or absence of one or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only;

or one or more polymorphisms which are in linkage disequilibrium with any one or more of these polymorphisms;

wherein the presence or absence of one or more of said polymorphisms is indicative of the subject's risk of developing one or more obstructive lung diseases selected from the group consisting of chronic obstructive pulmonary

disease (COPD), emphysema, or both COPD, emphysema, or both COPD and emphysema.

2. . A method according to claim 1 wherein the presence of one or more of the polymorphisms selected from the group consisting of:

the -765 CC or CG genotype in the promoter of the gene encoding COX2;

the +489 GG geneotype in the gene encoding TNFα;

the C89Y AA or AG geneotype in the gene encodoing SMAD3;

the 161 GG genotype in the gene encodoing MBL2;

the -1903 AA genotype in the gene encoding CMA1;

the Arg 197 Gln AA genotype in the gene encoding NAT2;

the -366 AA or AG genotype in the gene encoding ALOX5;

the HOM T2437C TT genotype in the gene encoding HSP 70;

the exon 1 +49 CT or TT genotype in the gene encoding Elafin; or

the -1607 1G1G or 1G2G genotype in the promoter of the gene encoding MMP1;

is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

3. A method according to claim 1 wherein the presence of one or more of the polymorphisms selected from the group consisting of:

the 105 AA genotype in the gene encoding IL18;

the -133 CC genotype in the promoter of the gene encoding IL18;

the -675 5G5G genotype in the promoter of the gene encoding PAI-1;

the 874 TT genotype in the gene encoding IFN–γ;

the +489 AA or AG genotype in the gene encoding TNFα;

the C89Y GG genotype in the gene encoding SMAD3;

the E469K GG genotype in the gene encoding ICAM1;

the Gly 881 Arg GC or CC genotype in the gene encoding NOD2;

the -366 GG genotype in the gene encoding ALOX5;

the HOM T2437C CC or CT genotype in the gene encoding HSP 70;

the +13924 AA genotype in the gene encoding CLCA1; or

the -159 CC genotype in the gene encoding CD-14;

is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

4. A method according to claim 1 wherein the method comprises analysing said sample for the presence or absence of one or more further polymorphisms selected from the group consisting of:

16 Arg/Gly in the gene encoding β2 adrenergic receptor (ADBR);

130 Arg/Gln (G/A) in the gene encoding Interleukin 13 (IL13);

298 Asp/Glu (T/G) in the gene encoding nitric oxide synthase 3 (NOS3);

Ile 105 Val (A/G) in the gene encoding glutathione S transferase P (GST-P);

Glu 416 Asp (T/G) in the gene encoding Vitamin D binding protein (VDBP);

Lys 420 Thr (A/C) in the gene encoding VDBP;

-1055 C/T in the promoter of the gene encoding IL13;

-308 G/A in the promoter of the gene encoding TNFα;

-511 A/G in the promoter of the gene encoding Interleukin 1B (IL1B);

Tyr 113 His T/C in the gene encoding Microsomal epoxide hydrolase (MEH);

Arg 139 G/A in the gene encoding MEH;

Gln 27 Glu C/G in the gene encoding ADBR

-1607 1G/2G in the promoter of the gene encoding MMP1 (with reference to the 2G allele only);

-1562 C/T in the promoter of the gene encoding MMP9;

M1 null in the gene encoding GST-1;

1237 G/A in the 3' region of the gene encoding α 1-antitrypsin;

-82 A/G in the promoter of the gene encoding MMP12;

T→C within codon 10 of the gene encoding TGFβ;

760 C/G in the gene encoding SOD3;

-1296 T/C within the promoter of the gene encoding TIMP3;

the S mutation in the gene encoding α1-antitrypsin; or

one or more polymorphisms which are in linkage disequilibrium with one or more of these polymorphisms.

5. A method according to claim 4 wherein the presence of one or more of the polymorphisms selected from the group consisting of:

the -765 CC or CG genotype in the promoter of the gene encoding COX2;

the 130 Arg/Gln AA genotype in the gene encoding IL13;

the 298 Asp/Glu TT genotype in the gene encoding NOS3;

the Lys 420 Thr AA or AC genotype in the gene encoding VDBP;

the Glu 416 Asp TT or TG genotype in the gene encoding VDBP;

the Ile 105 Val AA genotype in the gene encoding GSTP-1;

the MS genotype in the gene encoding α1-antitrypsin;

the +489 GG geneotype in the gene encoding TNFα;

the -308 GG geneotype in the gene encoding TNFα;

the C89Y AA or AG geneotype in the gene encodoing SMAD3;

the 161 GG genotype in the gene encodoing MBL2;

the -1903 AA genotype in the gene encoding CMA1;

the Arg 197 Gln AA genotype in the gene encoding NAT2;

the His 139 Arg GG genotype in the gene encoding MEH;

the -366 AA or AG genotype in the gene encoding ALOX5;

the HOM T2437C TT genotype in the gene encoding HSP 70;

the exon 1 +49 CT or TT genotype in the gene encoding Elafin;

the Gln 27 Glu GG genotype in the gene encoding ADBR; or

the -1607 1G1G or 1G2G genotype in the promoter of the gene encoding MMP1;

is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema

6. A method according to claim 4 or claim 5 wherein the presence of one or more of the polymorphisms selected from the group consisting of:

the 105 AA genotype in the gene encoding IL18;

the -133 CC genotype in the promoter of the gene encoding IL18;

the -675 5G5G genotype in the promoter of the gene encoding PAI-1;

the -1055 TT genotype in the promoter of the gene encoding IL13;

the 874 TT genotype in the gene encoding IFN–γ;

the +489 AA or AG genotype in the gene encoding TNFα;

the -308 AA or AG genotype in the gene encoding TNFα;

the C89Y GG genotype in the gene encoding SMAD3;

the E469K GG genotype in the gene encoding ICAM1;

the Gly 881 Arg GC or CC genotype in the gene encoding NOD2;

the -511 GG genotype in the gene encoding IL1B;

the Tyr 113 His TT genotype in the gene encoding MEH;

the -366 GG genotype in the gene encoding ALOX5;

the HOM T2437C CC or CT genotype in the gene encoding HSP 70;

the +13924 AA genotype in the gene encoding CLCA1; or

the -159 CC genotype in the gene encoding CD-14;

is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

7. A method of assessing a subject's risk of developing one or more obstructive lung diseases selected from COPD, emphysema, or both COPD and emphysema, said method comprising the steps:

(i) determining the presence or absence of at least one protective polymorphism associated with a reduced risk of developing COPD, emphysema, or both COPD and emphysema; and

(ii) in the absence of at least one protective polymorphisms, determining the presence or absence of at least one susceptibility polymorphism associated with an increased risk of developing COPD, emphysema, or both COPD and emphysema;

wherein the presence of one or more of said protective polymorphisms is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema, and the absence of at least one protective polymorphism in combination with the presence of at least one susceptibility polymorphism is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

8. A method according to claim 7 wherein said at least one protective polymorphism is selected from the group consisting of:

-765 C in the promoter of the gene encoding COX2;

130 Arg/Gln A in the gene encoding IL13;

298 Asp/Glu T in the gene encoding NOS3;

Lys 420 Thr A in the gene encoding VDBP;

Glu 416 Asp T in the gene encoding VDBP;

Ile 105 Val A in the gene encoding GSTP-1;

the S mutation in the gene encoding α1-antitrypsin;

+489 G in the gene encoding TNFα;

-308 G in the gene encoding TNFα;

C89Y A in the gene encoding SMAD3;

161 G in the gene encoding MBL2;

-1903 A in the gene encoding CMA1;

Arg 197 Gln A in the gene encoding NAT2;

His 139 Arg G in the gene encoding MEH;

-366 A in the gene encoding ALOX5;

HOM 2437 T in the gene encoding HSP 70;

exon 1 +49 T in the gene encodoing Elafin;

Gln 27 Glu G in the gene encoding ADBR; or

-1607 1G in the promoter of the gene encoding MMP1.

9. A method according to claim 7 wherein said at least one protective polymorphism is a genotype selected from the group consisting of:

the -765 CC or CG genotype in the promoter of the gene encoding COX2;

the 130 Arg/Gln AA genotype in the gene encoding IL13;

the 298 Asp/Glu TT genotype in the gene encoding NOS3;

the Lys 420 Thr AA or AC genotype in the gene encoding VDBP;

the Glu 416 Asp TT or TG genotype in the gene encoding VDBP;

the Ile 105 Val AA genotype in the gene encoding GSTP-1;

the MS genotype in the gene encoding α1-antitrypsin;

the +489 GG geneotype in the gene encoding TNFα;

the -308 GG geneotype in the gene encoding TNFα;

the C89Y AA or AG geneotype in the gene encodoing SMAD3;

the 161 GG genotype in the gene encodoing MBL2;

the -1903 AA genotype in the gene encoding CMA1;

the Arg 197 Gln AA genotype in the gene encoding NAT2;

the His 139 Arg GG genotype in the gene encoding MEH;

the -366 AA or AG genotype in the gene encoding ALOX5;

the HOM T2437C TT genotype in the gene encoding HSP 70;

the exon 1 +49 CT or TT genotype in the gene encoding Elafin;

the Gln 27 Glu GG genotype in the gene encoding ADBR; or

the -1607 1G1G or 1G2G genotype in the promoter of the gene encoding MMP1.

10. A method according to any one of claims 7 to 9 wherein said method comprises the additional step of determining the presence or absence of at least one further protective polymorphism selected from the group consisting of:

+760GG or +760CG within the gene encoding SOD3;

-1296TT within the promoter of the gene encoding TIMP3; or

CC (homozygous P allele) within codon 10 of the gene encoding TGFβ.

11. A method according to any one of claims 7 to 10 wherein said at least one susceptibility polymorphism is a genotype selected from the group consisting of:

105 AA in the gene encoding Interleukin 18;

-133 CC in the promoter of the gene encoding Interleukin 18;

-675 5G5G in the promoter of the gene encoding plasminogen activator inhibitor 1;

-1055 TT in the promoter of the gene encoding Interleukin 13;

874 AA in the gene encoding interferon–γ;

+489 AA or AG in the gene encoding TNFα;

-308 AA or AG in the gene encoding TNFα;

C89Y GG in the gene encoding SMAD3;

E469K GG in the gene encoding ICAM1;

Gly 881 Arg GC or CC in the gene encoding NOD2;

-511 GG in the gene encoding IL1B;

Tyr 113 His TT in the gene encoding MEH;

-366 GG in the gene encoding ALOX5;

HOM T2437C CC or CT in the gene encoding HSP 70;

+13924 AA in the gene encoding CLCA1; or

-159 CC in the gene encoding CD-14.

12. A method according to claim 11 wherein said method comprises the step of determining the presence or absence of at least one further susceptibility polymorphism selected from the group consisting of:

-82 AA within the promoter of the gene encoding MMP12;

-1607 2G2G within the promoter of the gene encoding MMP1;

-1562CT or -1562TT within the promoter of the gene encoding MMP9; or

1237AG or 1237AA (Tt or tt allele genotypes) within the 3' region of the gene encoding α 1-antitrypsin.

13. A method according to any one of claims 7 to 12 wherein the presence of two or more protective polymorphims irrespective of the presence of one or more susceptibility polymorphisms is indicative of reduced risk of developing COPD, emphysema, or both COPD and emphysema.
14. A method according to any one of claims 7 to 12 wherein in the absence of a protective polymorphism the presence of one or more susceptibility polymorphisms is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.
15. A method according to any one of claims 7 to 12 wherein the presence of two or more susceptibility polymorphisms is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.
16. A method of determining a subject's risk of developing chronic obstructive pulmonary disease (COPD) and/or emphysema, comprising analysing a sample from said subject for the presence of two or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding COX2;
105 C/A in the gene encoding IL18;
-133 G/C in the promoter of the gene encoding IL18;
-675 4G/5G in the promoter of the gene encoding PAI-1;
874 A/T in the gene encoding IFN-γ;
16Arg/Gly in the gene encoding ADBR;
130 Arg/Gln (G/A) in the gene encoding IL13;
298 Asp/Glu (T/G) in the gene encoding NOS3;
Ile 105 Val (A/G) in the gene encoding glutathione S transferase P (GST-P);
Glu 416 Asp (T/G) in the gene encoding VDBP;
Lys 420 Thr (A/C) in the gene encoding VDBP;
-1055 C/T in the promoter of the gene encoding IL13;
the S mutation in the gene encoding α1-antitrypsin;
+489 G/A in the gene encoding TNFα;
C89Y A/G in the gene encoding SMAD3;
E 469 K A/G in the gene encoding ICAM1;
Gly 881Arg G/C in the gene encoding NOD2;
161 G/A in the gene encoding MBL2;

-1903 G/A in the gene encoding CMA1;

Arg 197 Gln G/A in the gene encoding NAT2;

-366 G/A in the gene encoding ALOX5;

HOM T2437C in the gene encoding HSP 70;

+13924 T/A in the gene encoding CLCA1;

-159 C/T in the gene encoding CD-14;

exon 1 +49 C/T in the gene encoding Elafin;

-308 G/A in the promoter of the gene encoding TNFα;

-511 A/G in the promoter of the gene encoding IL1B;

Tyr 113 His T/C in the gene encoding MEH;

Arg 139 G/A in the gene encoding MEH;

Gln 27 Glu C/G in the gene encoding ADBR; or

-1607 1G/2G in the promoter of the gene encoding MMP1 (with reference to the 1G allele only).

17. A method according to any one of claims 1 to 16 wherein said method comprises the analysis of one or more epidemiological risk factors.

18. One or more nucleotide probes and/or primers for use in the method of any one of claims 1 to17 wherein the one or more nucleotide probes and/or primers span, or are able to be used to span, the polymorphic regions of the genes in which the polymorphism to be analysed is present.

19. A nucleic acid microarray which comprises a substrate presenting nucleic acid sequences capable of hybridizing to nucleic acid sequences which encode one or more of the polymorphisms selected from the group defined in claim 1 or sequences complimentary thereto.

20. A method of determining a subject's risk of developing COPD, emphysema, or both COPD and emphysema, said method comprising the steps:

(i) obtaining the result of one or more genetic tests of a sample from said subject; and (ii) analysing the result for the presence or absence of one or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only;

or one or more polymorphisms which are in linkage disequilibrium with any one or more of these polymorphisms;

wherein a result indicating the presence or absence of one or more of said polymorphisms is indicative of the subject's risk of developing COPD, emphysema, or both COPD and emphysema.

21. A method according to claim 20 wherein a result indicating the presence of one or more of the polymorphisms selected from the group consisting of:

the -765 CC or CG genotype in the promoter of the gene encoding COX2;

the +489 GG geneotype in the gene encoding TNFα;

the C89Y AA or AG geneotype in the gene encodoing SMAD3;

the 161 GG genotype in the gene encodoing MBL2;

the -1903 AA genotype in the gene encoding CMA1;

the Arg 197 Gln AA genotype in the gene encoding NAT2;

the -366 AA or AG genotype in the gene encoding ALOX5;

the HOM T2437C TT genotype in the gene encoding HSP 70;

the exon 1 +49 CT or TT genotype in the gene encoding Elafin; or

the -1607 1G1G or 1G2G genotype in the promoter of the gene encoding MMP1;

is indicative of a reduced risk of developing COPD, emphysema, or both COPD and emphysema.

22. A method according to claim 20 wherein a result indicating the presence of one or more of the polymorphisms selected from the group consisting of;

the 105 AA genotype in the gene encoding IL18;

the -133 CC genotype in the promoter of the gene encoding IL18;

the -675 5G5G genotype in the promoter of the gene encoding PAI-1;

the 874 TT genotype in the gene encoding IFN–γ;

the +489 AA or AG genotype in the gene encoding TNFα;

the C89Y GG genotype in the gene encoding SMAD3;

the E469K GG genotype in the gene encoding ICAM1;

the Gly 881 Arg GC or CC genotype in the gene encoding NOD2;

the -366 GG genotype in the gene encoding ALOX5;

the HOM T2437C CC or CT genotype in the gene encoding HSP 70;

the +13924 AA genotype in the gene encoding CLCA1; or

the -159 CC genotype in the gene encoding CD-14;

is indicative of an increased risk of developing COPD, emphysema, or both COPD and emphysema.

23. The use of at least one polymorphism in the assessment of a subject's risk of developing COPD, emphysema, or both COPD and emphysema, wherein said at least one polymorphism is selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only; or

one or more polymorphisms in linkage disequilibrium with any one of said polymorphisms.

24. The use according to claim 23, wherein said use is in conjunction with the use of at least one further polymorphism selected from the group consisting of:

16Arg/Gly in the gene encoding ADBR;

130 Arg/Gln (G/A) in the gene encoding IL13;

298 Asp/Glu (T/G) in the gene encoding NOS3;

Ile 105 Val (A/G) in the gene encoding GSTP;

Glu 416 Asp (T/G) in the gene encoding VDBP;

Lys 420 Thr (A/C) in the gene encoding VDBP;

-1055 C/T in the promoter of the gene encoding IL13;

the S mutation in the gene encoding α1-antitrypsin;

-308 G/A in the promoter of the gene encoding TNFα;

-511 A/G in the promoter of the gene encoding IL1B;

Tyr 113 His T/C in the gene encoding MEH;

His 139 Arg G/A in the gene encoding MEH;

Gln 27 Glu C/G in the gene encoding ADBR;

-1607 1G/2G in the promoter of the gene encoding MMP1;

-1562 C/T in the promoter of the gene encoding MMP9;

M1 (GSTM1) null in the gene encoding GST-1;

1237 G/A in the 3' region of the gene encoding α1-antitrypsin;

-82 A/G in the promoter of the gene encoding MMP12;

T→C within codon 10 of the gene encoding TGFβ;

760 C/G in the gene encoding SOD3;

-1296 T/C within the promoter of the gene encoding TIMP3; or

the S mutation in the gene encoding α1-antitrypsin.

25. A method treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema comprising the step of replicating, genotypically or phenotypically, the presence and/or functional effect of a protective polymorphism selected from the group defined in claim 8 in said subject.

26. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema, said subject having a detectable susceptibility polymorphism selected from the group defined in claim 11 which either upregulates or downregulates expression of a gene such that the physiologically active concentration of the expressed gene product is outside a range which is normal for the age and sex of the subject, said method comprising the step of restoring the physiologically active concentration of said product of gene expression to be within a range which is normal for the age and sex of the subject.

27. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the GG genotype at the -765 C/G polymorphism present in the promoter of the gene encoding COX2 has been determined, said method comprising administering to said subject an agent capable of reducing COX2 activity in said subject.

28. A method according to claim 27 wherein said agent is a COX2 inhibitor or a nonsteroidal anti-inflammatory drug (NSAID).

29. A method according to claim 28 wherein said COX2 inhibitor is selected from the group consisting of Celebrex (Celecoxib), Bextra (Valdecoxib), and Vioxx (Rofecoxib).

30. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the

AA genotype at the 105 C/A polymorphism in the gene encoding Interleukin 18 has been determined, said method comprising administering to said subject an agent capable of augmenting Interleukin 18 activity in said subject.

31. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the CC genotype at the -133 G/C polymorphism in the promoter of the gene encoding Interleukin 18 has been determined, said method comprising administering to said subject an agent capable of augmenting Interleukin 18 activity in said subject.
32. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the 5G5G genotype at the -675 4G/5G polymorphism in the promoter of the gene encoding plasminogen activator inhibitor 1 has been determined, said method comprising administering to said subject an agent capable of augmenting plasminogen activator inhibitor 1 activity in said subject.
33. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the AA genotype at the 874 A/T polymorphism in the gene encoding interferon-γ has been determined, said method comprising administering to said subject an agent capable of modulating interferon-γ activity in said subject.
34. A method of treating a subject having an increased risk of developing COPD, emphysema, or both COPD and emphysema and for whom the presence of the CC genotype at the -159 C/T polymorphism in the gene encoding CD-14 has been determined, said method comprising administering to said subject an agent capable of modulating CD-14 and/or IgE activity in said subject.
35. An antibody microarray which comprises a substrate presenting antibodies capable of binding to a product of expression of a gene the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism selected from the group defined in claim 1.
36. A method for screening for compounds that modulate the expression and/or activity of a gene, the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism selected from the group defined in claim 2 or claim 3, said method comprising the steps of:

contacting a candidate compound with a cell comprising a susceptibility or protective polymorphism selected from the group defined in claim 2 or claim 3 which has been determined to be associated with the upregulation or downregulation of expression of a gene; and

measuring the expression of said gene following contact with said candidate compound,

wherein a change in the level of expression after the contacting step as compared to before the contacting step is indicative of the ability of the compound to modulate the expression and/or activity of said gene.

37. A method according to claim 36 wherein said cell is a human lung cell which has been pre-screened to confirm the presence of said polymorphism.

38. A method according to claim 37 wherein said cell comprises a susceptibility polymorphism associated with downregulation of expression of said gene and said screening is for candidate compounds which upregulate expression of said gene.

39. A method according to claim 37 wherein said cell comprises a susceptibility polymorphism associated with downregulation of expression of said gene and said screening is for candidate compounds which upregulate expression of said gene.

40. A method according to claim 37 wherein said cell comprises a protective polymorphism associated with upregulation of expression of said gene and said screening is for candidate compounds which further upregulate expression of said gene.

41. A method according to claim 37 wherein said cell comprises a protective polymorphism associated with downregulation of expression of said gene and said screening is for candidate compounds which further downregulate expression of said gene.

42. A method for screening for compounds that modulate the expression and/or activity of a gene, the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism selected from the group defined in claim 2 or claim 3, said method comprising the steps of:

contacting a candidate compound with a cell comprising a gene, the expression of which is upregulated or downregulated when associated with a susceptibility or protective polymorphism selected from the group defined in

claim 2 or claim 3 but which in said cell the expression of which is neither upregulated nor downregulated; and

measuring the expression of said gene following contact with said candidate compound, wherein a change in the level of expression after the contacting step as compared to before the contacting step is indicative of the ability of the compound to modulate the expression and/or activity of said gene.

43. A method according to claim 42 wherein said cell is human lung cell which has been pre-screened to confirm the presence, and baseline level of expression, of said gene.

44. A method according to claim 43 wherein expression of the gene is downregulated when associated with a susceptibility polymorphism and said screening is for candidate compounds which in said cell, upregulate expression of said gene.

45. A method according to claim 43 wherein expression of the gene is upregulated when associated with a susceptibility polymorphism and said screening is for candidate compounds which, in said cell, downregulate expression of said gene.

46. A method according to claim 43 wherein expression of the gene is upregulated when associated with a protective polymorphism and said screening is for compounds which, in said cell, upregulate expression of said gene.

47. A method according to claim 43 wherein expression of the gene is downregulated when associated with a protective polymorphism and said screening is for compounds which, in said cell, downregulate expression of said gene.

48. A method of assessing the likely responsiveness of a subject having an increased risk of or suffering from COPD or emphysema to a prophylactic or therapeutic treatment, which treatment involves restoring the physiologically active concentration of a product of gene expression to be within a range which is normal for the age and sex of the subject, which method comprises detecting in said subject the presence or absence of a susceptibility polymorphism selected from the group defined in claim 3 which when present either upregulates or downregulates expression of said gene such that the physiological active concentration of the expressed gene product is outside said normal range, wherein the detection of the presence of said polymorphism is indicative of the subject likely responding to said treatment.

49. A kit for assessing a subject's risk of developing one or more obstructive lung diseases selected from COPD, emphysema, or both COPD and emphysema, said kit comprising a means of analysing a sample from said subject for the presence or absence of one or more polymorphisms selected from the group consisting of:

-765 C/G in the promoter of the gene encoding Cyclooxygenase 2 (COX2);

105 C/A in the gene encoding Interleukin18 (IL18);

-133 G/C in the promoter of the gene encoding IL18;

-675 4G/5G in the promoter of the gene encoding Plasminogen Activator Inhibitor 1 (PAI-1);

874 A/T in the gene encoding Interferon-γ (IFN-γ);

+489 G/A in the gene encoding Tissue Necrosis Factor α (TNFα);

C89Y A/G in the gene encoding SMAD3;

E 469 K A/G in the gene encoding Intracellular Adhesion molecule 1 (ICAM1);

Gly 881Arg G/C in the gene encoding Caspase (NOD2);

161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);

-1903 G/A in the gene encoding Chymase 1 (CMA1);

Arg 197 Gln G/A in the gene encoding N-Acetyl transferase 2 (NAT2);

-366 G/A in the gene encoding 5 Lipo-oxygenase (ALOX5);

HOM T2437C in the gene encoding Heat Shock Protein 70 (HSP 70);

+13924 T/A in the gene encoding Chloride Channel Calcium-activated 1 (CLCA1);

-159 C/T in the gene encoding Monocyte differentiation antigen CD-14 (CD-14);

exon 1 +49 C/T in the gene encoding Elafin;

-1607 1G/2G in the promoter of the gene encoding Matrix Metalloproteinase 1 (MMP1), with reference to the 1G allele only;

or one or more polymorphisms which are in linkage disequilibrium with any one or more of these polymorphisms.