"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 lung cancer in smokers and non-smokers using analysis of genetic polymorphisms and altered gene expression.

BACKGROUND OF THE INVENTION

Lung cancer is the second most common cancer and has been attributed primarily to cigarette smoking. Other factors contributing to the development of lung cancer include occupational exposure, genetic factors, radon exposure, exposure to other aero-pollutants and possibly dietary factors (Alberg AJ, et al., 2003). Non-smokers are estimated to have a one in 400 risk of lung cancer (0.25%). Smoking increases this risk by approximately 40 fold, such that smokers have a one in 10 risk of lung cancer (10%) and in long-term smokers the life-time risk of lung cancer has been reported to be as high 10-15% (Schwartz AG. 2004). Genetic factors are thought to play some part as evidenced by a weak familial tendency (among smokers) and the fact that only the minority of smokers get lung cancer. It is generally accepted that the majority of this genetic tendency comes from low penetrant high frequency polymorphisms, that is, polymorphisms which are common in the general population that in context of chronic smoking exposure contribute collectively to cancer development (Schwartz AG. 2004, Wu X et al., 2004). Several epidemiological studies have reported that impaired lung function (Anthonisen NR. 1989, Skillrud DM. 1986, Tockman MS et al., 1987, Kuller LH, et al., 1990, Nomura A, et al., 1991) or symptoms of obstructive lung disease (Mayne ST, et al., 1999) are independent risk factors for lung cancer and are possibly more relevant than smoking exposure dose.

Despite advances in the treatment of airways disease, current therapies do not significantly alter the natural history of lung cancer, which may include metastasis and progressive loss of lung function causing respiratory failure and death. Although cessation of smoking may be expected to reduce this decline in lung function, it is probable that if this is not achieved at an early stage, the loss is considerable and symptoms of worsening breathlessness likely cannot be averted. 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 lung cancer so that tests that identify at risk subjects can be developed and that new treatments can be discovered to reduce the adverse effects of lung cancer. The early diagnosis of lung cancer or of a propensity to developing lung cancer enables a broader range of prophylactic or therapeutic treatments to be employed than can be employed in the treatment of late stage lung cancer. Such prophylactic or early therapeutic treatment is also more likely to be successful, achieve remission, improve quality of life, and/or increase lifespan.

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 (TGFß); 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 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 lung cancer, or a risk of developing lung cancer-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 lung cancer than in control subjects. Analysis of these polymorphisms reveals an association between polymorphisms and the subject's risk of developing lung cancer.

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

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);or

-1082 A/G in the gene encoding Interleukin 10 (IL-10);

wherein the presence or absence of one or more of said polymorphisms is indicative of the subject's risk of developing lung cancer.

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:

Arg 399 Gln G/A in the X-ray repair complementing defective in Chinese hamster 1 (XRCC1) gene;

-251 A/T in the gene encoding Interleukin-8 (IL-8);

A870G in the gene encoding Cyclin D (CCND1);

-511 A/G in the gene encoding Interleukin 1B (IL-1B);

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the Xeroderma pigmentosum complementation group D (XPD) gene;

Ile 462 Val A/G in the gene encoding Cytochrome P450 1A1 (CYP1A1);

Ser 326 Cys G/C in the gene encoding 8-Oxoguanine DNA glycolase (OGG1);

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

1019 G/C Pst I in the gene encoding Cytochrome P450 2E1 (CYP2E1);

C/T Rsa I in the gene encoding Cytochrome P450 2E1;

GSTM null in the gene encoding Glutathione S-transferase M (GSTM);

-1607 1G/2G in the promoter of the gene encoding Matrix metalloproteinase 1 (MMP1);

Gln 185 Glu G/C in the gene encoding Nibrin (NBS1);

Phe 257 Ser C/T in the gene encoding REV1; or

Asp 148 Glu G/T in the gene encoding Apex nuclease (APE1).

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 Asp 298 Glu TT genotype in the gene encoding NOS3;

the Arg 312 Gln CG or GG genotype in the gene encoding SOD3;

the Asn 357 Ser AG or GG genotype in the gene encoding MMP12;

the 105 AC or CC genotype in the gene encoding IL-18;

the -133 CG or GG genotype in the gene encoding IL-18;

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

the -221 TT genotype in the gene encoding MUC5AC;

the intron 1 C/T TT genotype in the gene encoding Arg1;

the Leu252Val GG genotype in the gene encoding IGF2R;

the –1082 GG genotype in the gene encoding IL-10;

the -251 AA genotype in the gene encoding IL-8;

the Arg 399 Gln AA genotype in the XRCC1 gene ;

the A870G GG genotype in the gene encoding CCND1;

the -751 GG genotype in the promoter of the XPD gene ;

the Ile 462 Val AG or GG genotype in the gene encoding CYP1A1;

the Ser 326 Cys GG genotype in the gene encoding OGG1; or

the Phe 257 Ser CC genotype in the gene encoding REV1

may be indicative of a reduced risk of developing lung cancer.

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

the -786 TT genotype in the promoter of the gene encoding NOS3;

the Ala 15 Thr GG genotype in the gene encoding ACT;

the 105 AA genotype in the gene encoding IL-18;

the -133 CC genotype in the promoter of the gene encoding IL-18;

the 874 AA genotype in the gene encoding IFNγ;

the -765 GG genotype in the promoter of the gene encoding COX2;

the -447 CC or GC genotype in the gene encoding CTGF;

the +161 AA or AG genotype in the gene encoding MBL2;

the -511 GG genotype in the gene encoding IL-1B;

the A-670G AA genotype in the gene encoding FAS;

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

the Ile462 Val AA genotype in the gene encoding CYP1A1;

the 1019 G/C Pst I CC or CG genotype in the gene encoding CYP2E1;

the C/T Rsa I TT or TC genotype in the gene encoding CYP2E1;

the GSTM null genotype in the gene encoding GSTM;

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

the Gln 185 Glu CC genotype in the gene encoding NBS1; or

the Asp 148 Glu GG genotype in the gene encoding APE1;

may be indicative of an increased risk of developing lung cancer.

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 lung cancer (which can be termed "protective polymorphisms") and those associated with an increased risk of developing lung cancer (which can be termed "susceptibility polymorphisms").

Therefore, the present invention further provides a method of assessing a subject's risk of developing lung cancer, said method comprising:

determining the presence or absence of at least one protective polymorphism associated with a reduced risk of developing lung cancer; 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 lung cancer;

wherein the presence of one or more of said protective polymorphisms is indicative of a reduced risk of developing lung cancer, 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 lung cancer.

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

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

the Arg 312 Gln CG or GG genotype in the gene encoding SOD3;

the Asn 357 Ser AG or GG genotype in the gene encoding MMP12;

the 105 AC or CC genotype in the gene encoding IL-18;

the -133 CG or GG genotype in the gene encoding IL-18;

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

the -221 TT genotype in the gene encoding MUC5AC;

the intron 1 C/T TT genotype in the gene encoding Arg1;

the Leu252Val GG genotype in the gene encoding IGF2R;

the –1082 GG genotype in the gene encoding IL-10;

the -251 AA genotype in the gene encoding IL-8;

the Arg 399 Gln AA genotype in the XRCC1 gene ;

the A870G GG genotype in the gene encoding CCND1;

the -751 GG genotype in the promoter of the XPD gene ;

the Ile 462 Val AG or GG genotype in the gene encoding CYP1A1;

the Ser 326 Cys GG genotype in the gene encoding OGG1; or

the Phe 257 Ser CC genotype in the gene encoding REV1.

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

the -786 TT genotype in the promoter of the gene encoding NOS3;

the Ala 15 Thr GG genotype in the gene encoding ACT;

the 105 AA genotype in the gene encoding IL-18;

the -133 CC genotype in the promoter of the gene encoding IL-18;

the 874 AA genotype in the gene encoding IFNγ;

the -765 GG genotype in the promoter of the gene encoding COX2;

the -447 CC or GC genotype in the gene encoding CTGF;

the +161 AA or AG genotype in the gene encoding MBL2;

the -511 GG genotype in the gene encoding IL-1B;

the A-670G AA genotype in the gene encoding FAS;

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

the Ile462 Val AA genotype in the gene encoding CYP1A1;

the 1019 G/C Pst I CC or CG genotype in the gene encoding CYP2E1;

the C/T Rsa I TT or TC genotype in the gene encoding CYP2E1;

the GSTM null genotype in the gene encoding GSTM;

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

the Gln 185 Glu CC genotype in the gene encoding NBS1; or

the Asp 148 Glu GG genotype in the gene encoding APE1.

In a preferred form of the invention the presence of two or more protective polymorphisms is indicative of a reduced risk of developing lung cancer.

In a further preferred form of the invention the presence of two or more susceptibility polymorphisms is indicative of an increased risk of developing lung cancer.

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 lung cancer.

In another aspect, the invention provides a method of determining a subject's risk of developing lung cancer, 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:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);

-1082 A/G in the gene encoding Interleukin 10 (IL-10); or

one or more polymorphisms 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 lung cancer.

In a further aspect there is provided a method of determining a subject's risk of developing lung cancer comprising the analysis of two or more polymorphisms selected from the group consisting of:

Asp 298 Glu in the gene encoding NOS3;

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding SOD3;

-251 A/T in the gene encoding IL-8;

Ala 15 Thr in the gene encoding ACT;

Asn 357 Ser A/G in the gene encoding MMP12;

105 A/C in the gene encoding IL-18;

-133 G/C in the promoter of the gene encoding IL-18;

874 A/T in the gene encoding IFNγ;

Arg 399 Gln G/A in the XRCC1 gene;

A870G in the gene encoding CCND1;

-511 A/G in the gene encoding IL-1B;

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the XPD gene;

Ile 462 Val A/G in the gene encoding CYP1A1;

Ser 326 Cys G/C in the gene encoding OGG1;

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

1019 G/C Pst I in the gene encoding CYP2E1;

C/T Rsa I in the gene encoding CYP2E1;

GSTM null in the gene encoding GSTM;

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

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

-447 G/C in the gene encoding CTGF;

-221 C/T in the gene encoding MUC5AC;

+161 G/A in the gene encoding MBL2;

intron 1 C/T in the gene encoding Arg1;

Leu 252 Val C/G in the gene encoding IGF2R;

-1082 A/G in the gene encoding IL-10;

Gln 185 Glu G/C in the gene encoding NBS1;

Phe 257 Ser C/T in the gene encoding REV1;

Asp 148 Glu G/T in the gene encoding APE1; or

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

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 lung cancer.

The presence of asparagine at said position is indicative of reduced risk of developing lung cancer.

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 312 in the gene encoding SOD3.

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 15 in the gene encoding ACT.

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 357 in the gene encoding MMP12.

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 399 in the XRCC1 gene.

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 462 in the gene encoding CYP1A1.

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 326 in the gene encoding OGG1.

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 in 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 185 in the gene encoding NBS1.

The presence of glutamine at said position is indicative of an increased risk of developing lung cancer.

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 257 in the gene encoding REV1.

The presence of serine at said position is indicative of reduced risk of developing lung cancer.

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 252 in the gene encoding IGF2R.

The presence of valine at said position is indicative of reduced risk of developing lung cancer.

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 148 in the gene encoding APE1.

The presence of glutamate at said position is indicative of an increased risk of developing lung cancer.

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 lung cancer. Such epidemiological risk factors include but are not limited to smoking or exposure to tobacco smoke, age, sex, and familial history of lung cancer.

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 lung cancer, wherein said at least one polymorphism is selected from the group consisting of:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);

-1082 A/G in the gene encoding Interleukin 10 (IL-10); 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:

Arg 399 Gln G/A in the X-ray repair complementing defective in Chinese hamster 1 (XRCC1) gene;

-251 A/T in the gene encoding Interleukin-8 (IL-8);

A870G in the gene encoding Cyclin D (CCND1);

-511 A/G in the gene encoding Interleukin 1B (IL-1B);

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the Xeroderma pigmentosum complementation group D (XPD) gene;

Ile 462 Val A/G in the gene encoding Cytochrome P450 1A1 (CYP1A1);

Ser 326 Cys G/C in the gene encoding 8-Oxoguanine DNA glycolase (OGG1);

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

1019 G/C Pst I in the gene encoding Cytochrome P450 2E1 (CYP2E1);

C/T Rsa I in the gene encoding Cytochrome P450 2E1;

GSTM null in the gene encoding Glutathione S-transferase M (GSTM);

-1607 1G/2G in the promoter of the gene encoding Matrix metalloproteinase 1 (MMP1);

Gln 185 Glu G/C in the gene encoding Nibrin (NBS1);

Phe 257 Ser C/T in the gene encoding REV1;

Asp 148 Glu G/T in the gene encoding Apex nuclease (APE1);

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

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. Also provided are one or more nucleotide probes and/or primers comprising the sequence of any one of the probes and/or primers herein described, including any one comprising the sequence of any one of SEQ.ID.NO. 1 to 145, more preferably any one of SEQ.ID.NO. 3 to 142.

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 treating a subject having an increased risk of developing lung cancer 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 lung cancer, 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 a further aspect the present invention provides a method of treating a subject having an increased risk of developing lung cancer and for whom the presence of the AA genotype at the 105 C/A polymorphism in the gene encoding IL-18 has been determined, said method comprising administering to said subject an agent capable of augmenting IL-18 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 lung cancer and for whom the presence of the CC genotype at the -133 G/C polymorphism in the promoter of the gene encoding IL-18 has been determined, said method comprising administering to said subject an agent capable of augmenting IL-18 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, expression of the gene is downregulated when associated with a susceptibility polymorphism once said screening is for candidate compounds which in said cell, upregulate expression of said gene.

Preferably, said cell is a human lung cell which has been pre-screened to confirm the presence, and baseline level of 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 lung cancer 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 still a further aspect the present invention provides a method of treating a subject having an increased risk of developing lung cancer 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 yet still a further aspect the present invention provides a method of treating a subject having an increased risk of developing or cancer and for whom the presence of the 2G2G genotype at the -1607 1G/2G polymorphism in the promoter of the gene encoding MMP1 has been determined, said method comprising administering to said subject an agent capable of reducing MMP1 activity in said subject.

In one embodiment, said agent is an agent capable of increasing expression of or the activity of one or more tissue inhibitors of metalloproteinases (TIMPs), preferably the expression or activity of one or more of TIMP1, TIMP2, TIMP3, or TIMP4. In a further embodiment, said agent is an agent capable of reducing expression of or the activity of one or more membrane bound MMPs. In still a futher embodiment, said agent is a MMP inhibitor, preferably said MMP inhibitor is selected from the group comprising 4,5-dihydroxyanthaquinone-2-carboxylic acid (AQCA), anthraquinyl-mercaptoethyamine, anthraquinyl-alanine hydroxamate, and derivatives thereof.

In a further aspect, the present invention provides a kit for assessing a subject's risk of developing lung cancer, 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 FIGURES

Figure 1: depicts a graph showing the percentage of people with lung cancer plotted against the number of protective polymorphisms.

Figure 2: depicts a graph showing the percentage of people with lung cancer plotted against the number of susceptibility polymorphisms.

Figure 3 depicts a graph showing the frequency of protective polymorphisms in smokers with lung cancer and in resistant smokers.

Figure 4 depicts a graph showing the frequency of susceptibility polymorphisms in smokers with lung cancer and in resistant smokers.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Using case-control studies the frequencies of several genetic variants (polymorphisms) of candidate genes in smokers who have developed lung cancer 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 lung cancer (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.

In one embodiment described herein 19 susceptibility genetic polymorphisms and 17 protective genetic polymorphisms are identified. These are as follows:

Gene	Polymorphism	Role
Nitric Oxide synthase 3 (NOS3)	NOS3 Asp 298 Glu	TT protective
Nitric Oxide synthase 3 (NOS3)	NOS3 -786 T/C	TT susceptibility
Superoxide dismutase 3 (SOD3)	SOD3 Arg 312 Gln	CG/GG protective
XRCC1	XRCC1 Arg 399 Gln G/A	AA protective
Interleukin-8 (IL-8)	IL-8 -251 A/T	AA protective
Anti-chymotrypsin (ACT)	ACT Ala 15 Thr	GG susceptibility
Cyclin D (CCND1)	CCND1 A870G	GG protective AA susceptibility

Interleukin 1B (IL-1B)	IL-1B -511 A/G	GG susceptibility
FAS (Apo-1/CD95)	FAS A-670G	AA susceptibility
XPD	XPD -751 G/T	GG protective
CYP 1A1	CYP 1A1 Ile 462 Val A/G	GG/AG protective AA susceptibility
Matrix metalloproteinase 12 (MMP12)	MMP12 Asn 357 Ser A/G	GG/AG protective
8-Oxoguanine DNA glycolase (OGG1)	OGG1 Ser 326 Cys G/C	GG protective
N-acetyltransferase 2 (NAT2)	NAT2 Arg 197 Gln A/G	GG susceptibility
CYP2E1	CYP2E1 1019 G/C Pst I	CC/CG susceptibility
CYP2E1	CYP2E1 C/T Rsa I	TT/TC susceptibility
Interleukin -18 (IL-18)	IL-18 105 A/C	AC/CC protective AA susceptibility
Interleukin -18 (IL-18)	IL-18 -133 G/C	CG/GG protective CC susceptibility
Glutathione S-transferase M	GSTM null	Null susceptibility
Interferon gamma (IFNγ)	IFNγ 874 A/T	AA susceptibility
Cyclooxygenase 2 (COX2)	COX2 -765 C/G	CC/CG protective GG susceptibility
Matrix metalloproteinase 1 (MMP1)	MMP1 -1607 1G/2G	2G susceptibility
Connective tissue growth factor (CTGF)	CTGF -447 G/C	GC/CC susceptibility
Mucin 5AC (MUC5AC)	MUC5AC -221 C/T	TT protective
Mannose binding lectin 2 (MBL2)	MBL2 +161 G/A	AG/AA susceptibility
Nibrin (NBS1)	NBS1 Gln185Glu G/C	CC susceptibility
Arginase 1 (Arg1)	Arg1 intron 1 C/T	TT protective
REV1	REV1 Phe257Ser C/T	CC protective
Insulin-like growth factor II receptor (IGF2R)	IGF2R Leu252Val C/G	GG protective
Apex nuclease (Apex or APE1))	Apex Asp148Glu G/T	GG susceptibility
Interleukin 10 (IL-10)	IL-10 -1082 A/G	GG protective

A susceptibility genetic polymorphism is one which, when present, is indicative of an increased risk of developing lung cancer. In contrast, a protective genetic polymorphism is one which, when present, is indicative of a reduced risk of developing lung cancer.

As used herein, the phrase "risk of developing lung cancer" means the likelihood that a subject to whom the risk applies will develop lung cancer, and includes predisposition to, and potential onset of the disease. Accordingly, the phrase "increased risk of developing lung cancer" means that a subject having such an increased risk possesses an hereditary inclination or tendency to develop lung cancer. This does not mean that such a person will actually develop lung cancer at any time, merely that he or she has a greater likelihood of developing lung cancer compared to the general population of individuals that either does not possess a polymorphism associated with increased lung cancer or does possess a polymorphism associated with decreased lung

cancer risk. Subjects with an increased risk of developing lung cancer include those with a predisposition to lung cancer, such as a tendency or predilection regardless of their lung function at the time of assessment, for example, a subject who is genetically inclined to lung cancer 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 lung cancer if they keep smoking, and subjects with potential onset of lung cancer, who have a tendency to poor lung function on spirometry etc., consistent with lung cancer at the time of assessment.

Similarly, the phrase "decreased risk of developing lung cancer" means that a subject having such a decreased risk possesses an hereditary disinclination or reduced tendency to develop lung cancer. This does not mean that such a person will not develop lung cancer at any time, merely that he or she has a decreased likelihood of developing lung cancer compared to the general population of individuals that either does possess one or more polymorphisms associated with increased lung cancer, or does not possess a polymorphism associated with decreased lung cancer.

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 lung cancer may be diagnosed by analysing a sample from said subject for the presence of a polymorphism selected from the group consisting of:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);or

-1082 A/G in the gene encoding Interleukin 10 (IL-10).

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 lung cancer 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 lung cancer. 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 lung cancer and improve the ability to identify which smokers are at increased risk of developing lung cancer -related impaired lung function and lung cancer for predictive purposes.

The present results show for the first time that the minority of smokers who develop lung cancer 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 lung cancer. 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 described herein that have been reported to be in linkage disequilibrium are presented herein, and include the Interleukin-18 -133 C/G and 105 A/C polymorphisms, and the CYP2E1, 1019 G/C PstI and C/T RsaI polymorphisms, as shown below.

Gene	SNPs	rs numbers	Alleles in LD	LD between alleles	Phenotype in lung cancer
Interleukin-18	IL18 -133 C/G	rs360721	C allele	Strong LD	CC susceptibility
	IL18 105 A/C	rs549908	A allele		AA susceptibility
CYP2E1	CYP2E1 1019 G/C PstI	rs3813867	C allele	Strong LD	CG susceptibility
	CYP2E1 C/T RsaI	rs2031920	T allele		TC susceptibility

It will be apparent that polymorphsisms in linkage disequilibrium with one or more other polymorphism associated with increased or decreased risk of developing lung cancer will also provide utility as biomarkers for risk of developing lung cancer. 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 36.

It will also be apparent that frequently a variety of nomenclatures may exist for any given polymorphism. For example, the polymorphism referred to herein as Arg 312 Gln in the gene encoding SOD3 is believed to have been referred to variously as Arg 213 Gly, +760 G/C, and Arg 231 Gly (rs1799895). When referring to a susceptibility or protective polymorphism as herein described, such alternative nomenclatures are also contemplated by the present invention.

The methods of the invention are primarily directed to the detection and identification of the above polymorphisms associated with lung cancer, 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™ 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 lung cancer. Such risk factors include epidemiological risk factors associated with an increased risk of developing lung cancer. 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 lung cancer.

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 polymorphism 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 polymorphism 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 where a polymorphism 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 polymorphism 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 polymorphism is associated with decreased enzyme function, therapy can involve administration of active enzyme or an enzyme analogue to the subject. Similarly, where a polymorphism 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 protective polymorphism 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 polymorphism 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 lung cancer 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 polymorphisms 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 susceptibility 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 susceptibility polymorphisms; (b) upregulation of susceptibility genes that are normally downregulated in susceptibility polymorphisms; (c) downregulation of protective genes that are normally downregulated or not expressed (or null forms are expressed) in protective polymorphisms; and (d) upregulation of protective genes that are normally upregulated in protective polymorphisms. 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 susceptibility polymorphisms.

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.

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

EXAMPLE 1

Case Association Study

Introduction

Case-control association studies allow the careful selection of a control group where matching for important risk factors is critical. In this study, smokers diagnosed with lung cancer and smokers without lung cancer with normal lung function were compared. This unique control group is highly relevant as it is impossible to pre-select smokers with zero risk of lung cancer – i.e., those who although smokers will never develop lung cancer. Smokers with a high pack year history and normal lung function were used as a "low risk" group of smokers, as the Applicants believe it is not possible with current knowledge to identify a lower risk group of smokers. The Applicants believe, without wishing to be bound by any theory, that this approach allows for a more rigorous comparison of low penetrant, high frequency polymorphisms that may confer an increased risk of developing lung cancer. The Applicants also believe, again without wishing to be bound by any theory, that there may be polymorphisms that confer a degree of protection from lung cancer which may only be evident if a smoking cohort with normal lung function is utilised as a comparator group. Thus smokers with lung cancer would be expected to have a lower frequency of these polymorphisms compared to smokers with normal lung function and no diagnosed lung cancer.

Methods

Subject recruitment

Subjects of European decent who had smoked a minimum of fifteen pack years and diagnosed with lung cancer were recruited. Subjects met the following criteria: diagnosed with lung cancer based on radiological and histological grounds, including primary lung cancers with histological types of small cell lung cancer, squamous cell lung cancer, adenocarinoma of the lung, non-small cell cancer (where histological markers can not distinguish the subtype) and broncho-alveolar carcinoma. Subjects

could be of any age and at any stage of treatment after the diagnosis had been confirmed. One hundred and nine 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 or lung cancer in the past 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. 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 lung cancer sufferers and resistant smokers was found not to determine FEV or lung cancer.

This study shows that polymorphisms found in greater frequency in lung cancer patients compared to resistant smokers may reflect an increased susceptibility to the development of lung cancer. Similarly, polymorphisms found in greater frequency in resistant smokers compared to lung cancer may reflect a protective role.

Summary of characteristics for the lung cancer subjects and resistant smokers.

Parameter Median (IQR)	Lung Cancer N=109	Resistant smokers N=200	Differences
% male	52%	64%	ns
Age (yrs)	68 (11)	60 (12)	P<0.05
Pack years	40 (31)	43 (25)	P<0.05
Cigarettes/day	18 (11)	24(12)	ns
FEV1 (L)	1.7 (0.6)	2.8 (0.7)	P<0.05
FEV1 % predict	67 (22)	96% (10)	P<0.05
FEV1/FVC	59 (14)	82 (8)	P<0.05

Means and 95% confidence limits

Glutathione S-transferase null polymorphisms genotyping

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 [Cantlay AM. et al., 1994]. 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 KCl, 2.5 mM MgCl2 and 1.0 unit of Taq polymerase (Qiagen). Forward, internal (GSTM4) and reverse prime sequences were 5'-CTGCCCTACTTGATTGATGG-3' [SEQ.ID.NO.143], 5'-ATCTTCTCCTCTTCTGTCTC-3' [SEQ.ID.NO.144], and 5'-TTCTGGATTGTAGCAGATCA-3' [SEQ.ID.NO.145]. Cycling conditions consisted of 94C 60 s, 59C 30s, 72C 30 s for 35 cycles with an extended last extension of 3 min. Digested products were separated on 3% agarose gel. The products were visualised by ultraviolet transillumination following ethidium bromide staining and migration compared against a 1Kb plus ladder standard (Invitrogen). Genotypes were recorded in data spreadsheets and statistical analysis performed.

Cyclooxygenase 2 polymorphisms 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.

Matrix metalloproteinase 1 -1607 1G/2G polymorphisms genotyping

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 KCl, 1.5 mM $MgCl_2$ and 1.0 unit of Taq polymerase (Qiagen). Forward and reverse prime sequences were 5'-TCGTGAGAATGTCTTCCCATT-3' [SEQ.ID.NO.1] and 5'-TCTTGGATTGATTTGAGATAAGTGAAATC-3'[SEQ.ID.NO.2]. Cycling conditions consisted of 94°C 60 s, 55°C 30s, 72°C 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 1Kb plus ladder standard (Invitrogen). Genotypes were recorded in data spreadsheets and statistical analysis performed.

Polymorphism genotyping using the Sequenom Autoflex Mass Spectrometer

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 SequenomTM system (SequenomTM 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 MgCl2 1.25x, 25mM MgCl2 1.625mM, dNTP mix 25 mM 500uM, primers 4 uM 100nM, Taq polymerase (Quiagen 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. We used shrimp alkaline phosphotase (SAP) treatment (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

TERM	SNP_ID	2nd-PCRP	1st-PCRP
ACT	CYP2E1_1019 G/CPst1	ACGTTGGATGAAACCAGAGGGAAGCAAAGG [SEQ.ID.NO.3]	ACGTTGGATGTCATTGGTTGTGCTGCACCT [SEQ.ID.NO.4]
ACT	XPD -751 G/T	ACGTTGGATGCACCAGGAACCGTTTATGGC [SEQ.ID.NO.5]	ACGTTGGATGAGCAGCTAGAATCAGAGGAG [SEQ.ID.NO.6]
ACT	IL-18 105 A/C	ACGTTGGATGGTCAATGAAGAGAACTTGGTC [SEQ.ID.NO.7]	ACGTTGGATGAATGTTTATTGTAGAAAACC [SEQ.ID.NO.8]
ACT	IL-18 -133 G/C	ACGTTGGATGGGGTATTCATAAGCTGAAAC [SEQ.ID.NO.9]	ACGTTGGATGCCTTCAAGTTCAGTGGTCAG [SEQ.ID.NO.10]
ACT	CYP 1A1 Ile 462 Val	ACGTTGGATGGTGATTATCTTTGGCATGGG [SEQ.ID.NO.11]	ACGTTGGATGGGATAGCCAGGAAGAGAAAG [SEQ.ID.NO.12]
ACT	MMP12 Asn 357 Ser A/G	ACGTTGGATGCCCTATTTCTTTGTCTTCAC [SEQ.ID.NO.13]	ACGTTGGATGCTTGGGATAATTTGGCTCTG [SEQ.ID.NO.14]
ACT	OGG1 Ser 326 Cys G/C	ACGTTGGATGGGAACCCTTTCTGCGCTTTG [SEQ.ID.NO.15]	ACGTTGGATGCCTACAGGTGCTGTTCAGTG [SEQ.ID.NO.16]
ACT	NAT2 Arg 197 Gln A/G	ACGTTGGATGCCTGCCAAAGAAGAAACACC [SEQ.ID.NO.17]	ACGTTGGATGACGTCTGCAGGTATGTATTC [SEQ.ID.NO.18]
ACT	CYP2E1_C/T Rsa1	ACGTTGGATGGTTCTTAATTCATAGGTTGC [SEQ.ID.NO.19]	ACGTTGGATGCTTCATTTCTCATCATATTTTC [SEQ.ID.NO.20]
ACG	CCND1 A870G	ACGTTGGATGTAGGTGTCTCCCCCTGTAAG [SEQ.ID.NO.21]	ACGTTGGATGTCCTCTCCAGAGTGATCAAG [SEQ.ID.NO.22]
ACG	ILB1 -511 A/G	ACGTTGGATGATTTTCTCCTCAGAGGCTCC [SEQ.ID.NO.23]	ACGTTGGATGTGTCTGTATTGAGGGTGTGG [SEQ.ID.NO.24]
ACG	FAS_A-670G	ACGTTGGATGTTGTGGCTGCAACATGAGAG [SEQ.ID.NO.25]	ACGTTGGATGCTATGGCGCAACATCTGTAC [SEQ.ID.NO.26]
ACG	NOS3 -786 T/C	ACGTTGGATGACTGTAGTTTCCCTAGTCCC [SEQ.ID.NO.27]	ACGTTGGATGAGTCAGCAGAGAGACTAGGG [SEQ.ID.NO.28]
ACT	ACT_Ala15Thr	ACGTTGGATGGAGTTGAGAATGGAGAGAATG [SEQ.ID.NO.29]	ACGTTGGATGTCAAGTGGGCTGTTAGGGTG [SEQ.ID.NO.30]
ACT	SOD3 Arg 312 Gln	ACGTTGGATGTGCTGCGTGGTGGGCGTGTG [SEQ.ID.NO.31]	ACGTTGGATGGGCCTTGCACTCGCTCTCG [SEQ.ID.NO.32]
ACT	NOS3 Asp 298 Glu	ACGTTGGATGAAACGGTCGCTTCGACGTGC [SEQ.ID.NO.33]	ACGTTGGATGACCTCAAGGACCAGCTCGG [SEQ.ID.NO.34]
CGT	IL-8 -251 A/T	ACGTTGGATGACTGAAGCTCCACAATTTGG [SEQ.ID.NO.35]	ACGTTGGATGGCCACTCTAGTACTATATCTG [SEQ.ID.NO.36]
CGT	IFN gamma 874 A/T	ACGTTGGATGCAGACATTCACAATTGATTT [SEQ.ID.NO.37]	ACGTTGGATGGATAGTTCCAAACATGTGCG [SEQ.ID.NO.38]
ACT	XRCC1 Arg 399 Gln G/A	ACGTTGGATGTAAGGAGTGGGTGCTGGACT [SEQ.ID.NO.39]	ACGTTGGATGAGGATAAGGAGCAGGGTTGG [SEQ.ID.NO.40]

Sequenom conditions for the polymorphisms genotyping - 2

SNP_ID	AMP_LEN	UP_CONF	MP_CONF	Tm(NN)	PcGC	PWARN	UEP_DIR	UEP_MASS
CYP2E1_1019G/CPst1	119	95.2	71.3	46.7	47.1		F	5256.4
XPD -751 G/T	113	97.6	71.3	49.8	47.4		F	5689.7
IL-18 105 A/C	120	65.6	71.3	49.8	36.4		R	6702.4
IL-18 -133 G/C	112	93.5	81.3	47.1	42.1		F	5811.8
CYP 1A1 Ile 462 Val	102	98.2	81.3	55.6	55		F	6222.1
MMP12 Asn 357 Ser A/G	95	92.6	81.3	48	30.4		F	7070.6
OGG1 Ser 326 Cys G/C	99	96.5	82.2	58.9	70.6		R	5227.4
NAT2 Arg 197 Gln A/G	115	97.4	70	48.5	36.4		F	6635.3
CYP2E1_C/T Rsa1	105	62.8	77.8	46.4	26.1		R	7018.6
CCND1 A870G	106	98.1	83	45.8	47.1		R	5034.3
ILB1 -511 A/G	111	99.2	83	46	47.1		R	5203.4
FAS_A-670G	103	99.2	83	54.4	50		R	6166
NOS3 -786 T/C	114	97.5	83	61.8	61.9		F	6358.1
ACT_Ala15Thr	118	93.4	68.2	45.2	47.1		F	5136.4
SOD3 Arg 312 Gln	119	63.2	68.2	55.5	57.9		F	5855.8
NOS3 Asp 298 Glu	113	82.2	68.2	65.4	66.7		F	6432.2
IL-8 -251 A/T	119	92.6	75.8	45.9	28.6		R	6428.2
IFN gamma 874 A/T	112	75.3	75.8	46.4	26.1		F	6943.6
XRCC1 Arg 399 Gln G/A	109	93.6	93.6	66.8	82.4		F	5099.3

Sequenom conditions for the polymorphisms genotyping - 3

SNP_ID	UEP_SEQ	EXT1_CALL	EXT1_MASS	EXT1_SEQ	EXT2_CALL
CYP2E1_1019G/CPst1	TTCTTGGTTCAGGAGAG [SEQ.ID.NO.41]	C	5529.6	TTCTTGGTTCAGGAGAGC [SEQ.ID.NO.42]	G
XPD -751 G/T	GCAATCTGCTCTATCCTCT [SEQ.ID.NO.43]	T	5977.9	GCAATCTGCTCTATCCTCTT [SEQ.ID.NO.44]	G
IL-18 105 A/C	ATTCAAGCTTGCCAAAGTAATC [SEQ.ID.NO.45]	A	6990.6	ATTCAAGCTTGCCAAAGTAATCT [SEQ.ID.NO.46]	C
IL-18 -133 G/C	CATAAGCTGAAACTTCTGG [SEQ.ID.NO.47]	C	6085	CATAAGCTGAAACTTCTGGC [SEQ.ID.NO.48]	G
CYP 1A1 Ile 462 Val	GGAAGTGTATCGGTGAGACC [SEQ.ID.NO.49]	A	6519.3	GGAAGTGTATCGGTGAGACCA [SEQ.ID.NO.50]	G
MMP12 Asn 357 Ser A/G	TGACAAATACTGGTTAATTAGCA [SEQ.ID.NO.51]	A	7367.8	TGACAAATACTGGTTAATTAGCAA [SEQ.ID.NO.52]	G
OGG1 Ser 326 Cys G/C	GCTCCTGAGCATGGCGG [SEQ.ID.NO.53]	G	5500.6	GCTCCTGAGCATGGCGGC [SEQ.ID.NO.54]	C
NAT2 Arg 197 Gln A/G	TACTTATTTACGCTTGAACCTC [SEQ.ID.NO.55]	A	6932.5	TACTTATTTACGCTTGAACCTCA [SEQ.ID.NO.56]	G
CYP2E1_C/T Rsa1	CTTAATTCATAGGTTGCAATTTT [SEQ.ID.NO.57]	T	7315.8	CTTAATTCATAGGTTGCAATTTTA [SEQ.ID.NO.58]	C
CCND1 A870G	ACATCACCCTCACTTAC [SEQ.ID.NO.59]	G	5307.5	ACATCACCCTCACTTACC [SEQ.ID.NO.60]	A
ILB1 -511 A/G	AATTGACAGAGAGCTCC [SEQ.ID.NO.61]	G	5476.6	AATTGACAGAGAGCTCCC [SEQ.ID.NO.62]	A
FAS_A-670G	ATGAGAGGCTCACAGACGTT [SEQ.ID.NO.63]	G	6439.2	ATGAGAGGCTCACAGACGTTC [SEQ.ID.NO.64]	A
NOS3 -786 T/C	GGCATCAAGCTCTTCCCTGGC [SEQ.ID.NO.65]	C	6631.3	GGCATCAAGCTCTTCCCTGGCC [SEQ.ID.NO.66]	T
ACT_Ala15Thr	GAATGTTACCTCTCCTG [SEQ.ID.NO.67]	A	5433.6	GAATGTTACCTCTCCTGA [SEQ.ID.NO.68]	G
SOD3 Arg 312 Gln	GCACTCAGAGCGCAAGAAG [SEQ.ID.NO.69]	C	6129	GCACTCAGAGCGCAAGAAGC [SEQ.ID.NO.70]	G
NOS3 Asp 298 Glu	GCTGCTGCAGGCCCCAGATGA [SEQ.ID.NO.71]	T	6720.4	GCTGCTGCAGGCCCCAGATGAT [SEQ.ID.NO.72]	G
IL-8 -251 A/T	CACAATTTGGTGAATTATCAA [SEQ.ID.NO.73]	A	6716.4	CACAATTTGGTGAATTATCAAT [SEQ.ID.NO.74]	T
IFN gamma 874 A/T	TTCTTACAACACAAAATCAAATC [SEQ.ID.NO.75]	T	7231.8	TTCTTACAACACAAAATCAAATCT [SEQ.ID.NO.76]	A
XRCC1 Arg 399 Gln G/A	TCGGCGGCTGCCCTCCC [SEQ.ID.NO.77]	A	5396.5	TCGGCGGCTGCCCTCCCA [SEQ.ID.NO.78]	G

Sequenom conditions for the polymorphisms genotyping - 4

SNP_ID	EXT2_MASS	EXT2_SEQ	1stPAUSE
CYP2E1_1019G/CPst1	5873.8	TTCTTGGTTCAGGAGAGGT [SEQ.ID.NO.79]	5585.6
XPD -751 G/T	6292.1	GCAATCTGCTCTATCCTCTGC [SEQ.ID.NO.80]	6018.9
IL-18 105 A/C	7658	ATTCAAGCTTGCCAAAGTAATCGGA [SEQ.ID.NO.81]	7031.6
IL-18 -133 G/C	6438.2	CATAAGCTGAAACTTCTGGGA [SEQ.ID.NO.82]	6141
CYP 1A1 Ile 462 Val	6839.5	GGAAGTGTATCGGTGAGACCGT [SEQ.ID.NO.83]	6551.3
MMP12 Asn 357 Ser A/G	7688	TGACAAATACTGGTTAATTAGCAGT [SEQ.ID.NO.84]	7399.8
OGG1 Ser 326 Cys G/C	5853.8	GCTCCTGAGCATGGCGGGA [SEQ.ID.NO.85]	5556.6
NAT2 Arg 197 Gln A/G	7261.8	TACTTATTTACGCTTGAACCTCGA [SEQ.ID.NO.86]	6964.5
CYP2E1_C/T Rsa1	7636	CTTAATTCATAGGTTGCAATTTTGT [SEQ.ID.NO.87]	7347.8
CCND1 A870G	5651.7	ACATCACCCTCACTTACTG [SEQ.ID.NO.88]	5338.5
ILB1 -511 A/G	5820.8	AATTGACAGAGAGCTCCTG [SEQ.ID.NO.89]	5507.6
FAS_A-670G	6743.4	ATGAGAGGCTCACAGACGTTTC [SEQ.ID.NO.90]	6470.2
NOS3 -786 T/C	6975.5	GGCATCAAGCTCTTCCCTGGCTG [SEQ.ID.NO.91]	6662.3
ACT_Ala15Thr	5738.7	GAATGTTACCTCTCCTGGC [SEQ.ID.NO.92]	5465.6
SOD3 Arg 312 Gln	7116.6	GCACTCAGAGCGCAAGAAGGGGC [SEQ.ID.NO.93]	6185
NOS3 Asp 298 Glu	7034.6	GCTGCTGCAGGCCCCAGATGAGC [SEQ.ID.NO.94]	6761.4
IL-8 -251 A/T	7029.6	CACAATTTGGTGAATTATCAAAT [SEQ.ID.NO.95]	6741.4
IFN gamma 874 A/T	7530	TTCTTACAACACAAAATCAAATCAC [SEQ.ID.NO.96]	7256.8
XRCC1 Arg 399 Gln G/A	6054.9	TCGGCGGCTGCCCTCCCGGA [SEQ.ID.NO.97]	5428.5

Sequenom conditions for the polymorphisms genotyping - 5

TERM	SNP_ID	2nd-PCRP	1st-PCRP
ACT	CTGF-447G/C	ACGTTGGATGAGGTAGCTGAAGAGGCAAAC [SEQ.ID.NO.98]	ACGTTGGATGGCCTATAGCCTCTAAAACGC [SEQ.ID.NO.99]
ACT	NBS1 Gln185Glu G/C	ACGTTGGATGCTTTCAATTTGTGGAGGCTG [SEQ.ID.NO.100]	ACGTTGGATGTGTGCACTCATTTGTGGACG [SEQ.ID.NO.101]
ACT	MBL2 161 G/A	ACGTTGGATGGTAGCTCTCCAGGCATCAAC [SEQ.ID.NO.102]	ACGTTGGATGGTACCTGGTTCCCCCTTTTC [SEQ.ID.NO.103]
ACT	IGF2R Leu252Val C/G	ACGTTGGATGACACCAGGCGTTTGATGTTG [SEQ.ID.NO.104]	ACGTTGGATGAAAAACGCCAACAGCATCGG [SEQ.ID.NO.105]
ACT	MUC5AC -221 C/T	ACGTTGGATGAGGCGGAGATGGGTGTGTC [SEQ.ID.NO.106]	ACGTTGGATGAGTCTAGGGTGGGGTATGTG [SEQ.ID.NO.107]
ACT	Arg1 intron1 C/T	ACGTTGGATGATGTGTGGATTCACAGCTCG [SEQ.ID.NO.108]	ACGTTGGATGGGGTTGGCAACTCTAAAAGG [SEQ.ID.NO.109]
ACT	REV1 Phe257Ser C/T	ACGTTGGATGCTCTGAAATCAGTGCTGCTC [SEQ.ID.NO.110]	ACGTTGGATGATGGTCAACAGTGTTGCCAG [SEQ.ID.NO.111]
ACT	Apex1 Asp148Glu G/T	ACGTTGGATGCACCTCTTGATTGCTTTCCC [SEQ.ID.NO.112]	ACGTTGGATGACCCGGCCTTCCTGATCATG [SEQ.ID.NO.113]
ACG	IL-10 -1082 A/G	ACGTTGGATGATTCCATGGAGGCTGGATAG [SEQ.ID.NO.114]	ACGTTGGATGGACAACACTACTAAGGCTTC [SEQ.ID.NO.115]

Sequenom conditions for the polymorphisms genotyping - 6

SNP_ID	AMP_LEN	UP_CONF	MP_CONF	Tm(NN)	PcGC	PWARN	UEP_DIR	UEP_MASS
CTGF-447G/C	119	98.2	65	52	52.9		R	5090.3
NBS1 Gln185Glu G/C	118	97	65	51	52.9		R	5192.4
MBL2 161 G/A	99	96.8	65	50.3	52.9		F	5299.5
IGF2R Leu252Val C/G	114	98.5	67.8	68.6	82.4		F	5206.4
MUC5AC -221 C/T	119	81.8	67.8	56.9	64.7	g	R	5273.4
Arg1 intron1 C/T	102	99.6	67.8	53.3	52.6		R	5932.9
REV1 Phe257Ser C/T	105	99.6	67.8	57.5	55		R	6003.9
Apex1 Asp148Glu G/T	114	92.9	67.8	46.8	35		F	6113
IL-10 -1082 A/G	107	98	68.8	51.2	58.8		R	4977.2

Sequenom conditions for the polymorphisms genotyping - 7

SNP_ID	UEP_SEQ	EXT1_CALL	EXT1_MASS	EXT1_SEQ
CTGF-447G/C	AAAAGGTTTCTCCCCCC [SEQ.ID.NO.116]	G	5363.5	AAAAGGTTTCTCCCCCCC [SEQ.ID.NO.117]
NBS1 Gln185Glu G/C	AGGCTGCTTCTTGGACT [SEQ.ID.NO.118]	G	5465.6	AGGCTGCTTCTTGGACTC [SEQ.ID.NO.119]
MBL2 161 G/A	CAAAGATGGGCGTGATG [SEQ.ID.NO.120]	A	5596.7	CAAAGATGGGCGTGATGA [SEQ.ID.NO.121]
IGF2R Leu252Val C/G	GCCAGCCCCGGGACGGA [SEQ.ID.NO.122]	C	5479.6	GCCAGCCCCGGGACGGAC [SEQ.ID.NO.123]
MUC5AC -221 C/T	ATGGGTGTGTCTGCCGG [SEQ.ID.NO.124]	T	5570.6	ATGGGTGTGTCTGCCGGA [SEQ.ID.NO.125]
Arg1 intron1 C/T	GGCTGTAAGGAAATCTGGG [SEQ.ID.NO.126]	T	6230.1	GGCTGTAAGGAAATCTGGGA [SEQ.ID.NO.127]
REV1 Phe257Ser C/T	CCTTATCCTCCTCCTGGGAA [SEQ.ID.NO.128]	T	6301.1	CCTTATCCTCCTCCTGGGAAA [SEQ.ID.NO.129]
Apex1 Asp148Glu G/T	TGTTTCATTTCTATAGGCGA [SEQ.ID.NO.130]	T	6401.2	TGTTTCATTTCTATAGGCGAT [SEQ.ID.NO.131]
IL-10 -1082 A/G	CCTATCCCTACTTCCCC [SEQ.ID.NO.132]	G	5250.4	CCTATCCCTACTTCCCCC [SEQ.ID.NO.133]

Sequenom conditions for the polymorphisms genotyping - 8

SNP_ID	EXT2_CALL	EXT2_MASS	EXT2_SEQ	1stPAUSE
CTGF-447G/C	C	5716.7	AAAAGGTTTCTCCCCCCGA [SEQ.ID.NO.134]	5419.5
NBS1 Gln185Glu G/C	C	5818.8	AGGCTGCTTCTTGGACTGA [SEQ.ID.NO.135]	5521.6
MBL2 161 G/A	G	5901.9	CAAAGATGGGCGTGATGGC [SEQ.ID.NO.136]	5628.7
IGF2R Leu252Val C/G	G	5823.8	GCCAGCCCCGGGACGGAGT [SEQ.ID.NO.137]	5535.6
MUC5AC -221 C/T	C	5890.8	ATGGGTGTGTCTGCCGGGT [SEQ.ID.NO.138]	5602.6
Arg1 intron1 C/T	C	6879.5	GGCTGTAAGGAAATCTGGGGGT [SEQ.ID.NO.139]	6262.1
REV1 Phe257Ser C/T	C	6630.3	CCTTATCCTCCTCCTGGGAAGA [SEQ.ID.NO.140]	6333.1
Apex1 Asp148Glu G/T	G	7068.6	TGTTTCATTTCTATAGGCGAGGA [SEQ.ID.NO.141]	6442.2
IL-10 -1082 A/G	A	5858.8	CCTATCCCTACTTCCCCTTC [SEQ.ID.NO.142]	5281.4

RESULTS

Table 1a. Nitric oxide synthase 3 Asp 298 Glu (T/G) polymorphism allele and genotype frequencies in the lung cancer 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%)
Lung Cancer n=107 (%)	71 (33%)	143 (67%)	9 (8%)	53 (50%)	45 (42%)
Resistant n=198 (%)	135 (34%)	261 (66%)	28[1, 2] (14%)	79 (40%)	91 (46%)

* number of chromosomes (2n)

1. Genotype. TT vs TG/GG for resistant vs lung cancer, Odds ratio (OR) =1.8, 95% confidence limits 0.8-4.3, χ^2 (Yates uncorrected)= 2.14, p=0.14,
   TT genotype = protective for lung cancer
2. Genotype. TT vs TG/GG for resistant vs controls, Odds ratio (OR) =2.2, 95% confidence limits 1.0-4.6, χ^2 (Yates corrected)= 4.2, p=0.04,
   TT genotype = protective for lung cancer

Table 1b. Nitric oxide synthase 3 -786 T/C polymorphism allele and genotype frequencies in the lung cancer patients, resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
Controls n=183 (%)					
Lung Cancer n=107 (%)	82 (38%)	132 (62%)	16 (15%)	50 (47%)	41[1] (38%)
Resistant n=198 (%)	166 (42%)	228 (58%)	31 (16%)	104 (53%)	62 (31%)

* number of chromosomes (2n)

1. Genotype. TT vs CC/CT for Lung cancer vs resistant, Odds ratio (OR) =1.4, 95% confidence limits 0.8-2.3, χ^2 (Yates uncorrected)= 1.45, p=0.23,
   TT genotype = susceptibility to lung cancer

Table 2. Super oxide dismutase 3 Arg 312 Gln C/G polymorphism allele and genotype frequencies in the lung cancer, resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	G	CC	CG	GG
Controls n=190 (%)	371 (98%)	9 (2%)	183 (96%)	5 (3%)	2 (1%)
Lung Cancer n=104 (%)	208 (100%)	0 (0%)	104 (100%)	0 (0%)	0 (0%)
Resistant n=182 (%)	390 (98%)	10 (3%)	191 (95%)	8[1] (4%)	1[1] (1%)

* number of chromosomes (2n)

1. Genotype CG/GG vs CC for resistant vs lung cancer, Yates uncorrected=3.38, P=0.07 and Fisher's Two tailed test, P=0.03.
   CG/GG = protective for lung cancer

Table 3. XRCC1 Arg 399 Gln A/G polymorphism allele and genotype frequencies in the lung cancer, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Controls n=190 (%)					
Lung Cancer n=103 (%)	68 (33%)	138 (67%)	4 (4%)	60 (58%)	39 (38%)
Resistant n=193 (%)	132 (34%)	254 (66%)	18[1] (9%)	96 (50%)	79 (41%)

* number of chromosomes (2n)

1. Genotype. AA vs AG/GG for resistant vs lung cancer, Odds ratio (OR) = 2.6, 95% confidence limits 0.8-9.2, χ^2 (Yates uncorrected)= 2.89, p=0.09.
   AA genotype = protective for lung cancer

Table 4. Interleukin 8 -251 A/T polymorphism allele and genotype frequencies in the lung cancer, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	T	AA	AT	TT
Controls n=188 (%)	175 (47%)	201 (53%)	39 (21%)	97 (52%)	52 (28%)
Lung Cancer n=90	68 (38%)	112 (62%)	6 (7%)	56 (52%)	28 (31%)
Resistant n=199 (%)	192[2] (48%)	206 (52%)	45[1] (23%)	102 (51%)	52 (26%)

* number of chromosomes (2n)

1. Genotype. AA vs AT/TT for resistant vs lung cancer, Odds ratio (OR) =4.1, 95% confidence limits=1.6=11.2, χ^2 (Yates corrected)=9.8, p=0.002,
   AA = protective for lung cancer
2. Allele. A vs T for resistant smokers vs lung cancer, Odds ratio (OR) =1.5, 95% confidence limits 1.0-2.2, χ^2 (Yates corrected)= 5.07, p=0.02
   A = protective for lung cancer

Table 5. Anti-chymotrypsin Ala -15 Thr polymorphism allele and genotype frequencies in the lung cancer subjects, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Lung Cancer n=108	99 (46%)	117[2] (54%)	24 (22%)	51 (47%)	33[1] (31%)
Resistant n=196 (%)	207 (53%)	185 (47%)	52 (27%)	103 (53%)	41 (21%)

* number of chromosomes (2n)

1. Genotype. GG vs AA/AG for Lung cancer vs resistant, Odds ratio (OR) = 1.7, 95% confidence limits= 0.9-2.9, χ^2 (Yates uncorrected)=3.51, p=0.06, GG = susceptibile to lung cancer
2. Allele. G vs A for lung cancer vs resistant smokers, Odds ratio (OR) =1.3, 95% confidence limits 0.9-1.9, χ^2 (Yates uncorrected)= 2.71, p=0.10, G = susceptibility to lung cancer

Table 6. Cyclin D1 (CCND1) A 870 G polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Lung Cancer n=107	109 (51%)	105 (49%)	25[2] (23%)	59 (55%)	23 (21%)
Resistant n=199 (%)	188 (47%)	210 (53%)	45 (23%)	98 (49%)	56[1] (28%)

* number of chromosomes (2n)

1. Genotype. GG vs AG/AA for Resistant vs lung cancer, Odds ratio (OR) =1.4, 95% confidence limits=0.8-2.6, χ^2 (Yates uncorrected)=1.6, p=0.20, GG = protective for lung cancer
2. Genotype. AG/AA vs GG for Lung cancer vs resistant, Odds ratio (OR) =1.4, 95% confidence limits=0.8-2.6, χ^2 (Yates uncorrected)=1.6, p=0.20, AA = susceptibility to lung cancer

Table 7. Interleukin 1B (IL-1b) -511 A/G polymorphism allele and genotype frequencies in the lung cancer subjects, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Lung Cancer n=107	64 (30%)	150 (70%)	12 (11%)	40 (37%)	55[1] (51%)
Resistant n=198 (%)	143 (36%)	253 (64%)	23 (12%)	97 (49%)	78 (39%)

* number of chromosomes (2n)

1. Genotype. GG vs AA/AG for Lung cancer vs resistant, Odds ratio (OR) = 1.6, 95% confidence limits= 1-2.7, χ^2 (Yates uncorrected)= 4.07, p=0.04, GG = susceptibility to lung cancer

Table 8. FAS (Apo-1/CD 95) A -670 G polymorphism allele and genotype frequencies in the lung cancer subjects, resistant smokers and controls.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG

Lung Cancer n=106	121[2] (57%)	91 (43%)	32[1] (30%)	57 (54%)	17 (16%)
Resistant n=198 (%)	202 (51%)	194 (49%)	45 (23%)	112 (57%)	41 (21%)

* number of chromosomes (2n)

1. Genotype. AA vs AG/GG for Lung cancer vs resistant, Odds ratio (OR) =1.5, 95% confidence limits=0.8-2.6, χ^2 (Yates uncorrected)=2.03, p=0.15, AA =susceptibility to lung cancer
2. Allele. A vs G for Lung cancer vs resistant, Odds ratio (OR) =1.3, 95% confidence limits 0.9-1.8, χ^2 (Yates uncorrected)= 2.04, p=0.15, A = susceptibility to lung cancer

Table 9. XPD 751 T/G polymorphism allele and genotype frequencies in the lung cancer subjects, resistant smokers and controls.

Frequency	Allele*		Genotype		
	G	T	GG	TG	TT
Lung Cancer n=108	72 (33%)	144 (66%)	11 (10%)	50 (46%)	47 (44%)
Resistant n=197 (%)	147 (37%)	247 (63%)	31[1] (16%)	85 (43%)	81 (41%)

* number of chromosomes (2n)

1. Genotype. GG vs TG/TT for Resistant vs lung cancer, Odds ratio (OR) =1.7, 95% confidence limits= 0.8-3.7, χ^2 (Yates uncorrected)= 1.81, p=0.18, GG = protective for lung cancer

Table 10. Cytochrome P450 1A1 Ile 462 Val G/A polymorphism allele and genotype frequencies in the lung cancer subjects, resistant smokers and controls.

Frequency	Allele*		Genotype		
	G	A	GG	AG	AA
Lung Cancer n=109	5 (2%)	213 (98%)	0 (0%)	5 (5%)	104[1] (95%)
Resistant n=199 (%)	20 (5%)	378 (95%)	13[1] (1%)	18[1] (9%)	180 (90%)

* number of chromosomes (2n)

1. Genotype. AG/GG vs AA for Resistant vs lung cancer, Odds ratio (OR) =2.2, 95% confidence limits= 0.7-6.9, χ^2 (Yates uncorrected)=2.41, p=0.12, GG/AG = protective for lung cancer, AA = susceptibility to lung cancer

Table 11. MMP12 Asn 357 Ser polymorphism allele and genotype frequencies in the lung cancer subjects, resistant smokers and controls.

Frequency	Allele*		Genotype		
	G	A	GG	AG	AA

Lung Cancer n=109	8 (4%)	210 (96%)	1 (1%)	6 (5%)	102 (94%)
Resistant n=199 (%)	21 (5%)	377 (95%)	0[1] (0%)	21[1] (11%)	178 (89%)

* number of chromosomes (2n)

1. Genotype. GG/AG vs AA for Resistant vs lung cancer, Odds ratio (OR) =1.7, 95% confidence limits= 0.7-4.6, χ^2 (Yates uncorrected)=1.45, p=0.23, GG/AG = protective for lung cancer

Table 12. 8-oxoguanine DNA glycosylase (OGG1) Ser 326 Cys C/G polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	G	C	GG	CG	CC
Lung Cancer n=109	40 (18%)	178 (82%)	2 (2%)	36 (33%)	71 (65%)
Resistant n=199 (%)	100 (25%)	298 (75%)	14[1] (7%)	72 (36%)	113 (57%)

* number of chromosomes (2n)

1. Genotype. GG vs CG/CC for Resistant vs lung cancer, Odds ratio (OR) =4.0, 95% confidence limits=0.9-26.3, χ^2 (Yates uncorrected)=3.87, p=0.05, GG = protective for lung cancer

Table 13. N-Acetyltransferase 2 Arg 197 Gln G/A polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	A	G	AA	AG	GG
Lung Cancer n=106	55 (26%)	157 (74%)	9 (8%)	37 (35%)	60[1] (57%)
Resistant n=195 (%)	122 (31%)	268 (69%)	17 (9%)	88 (45%)	90 (46%)

* number of chromosomes (2n)

1. Genotype. GG vs AG/AA for Lung cancer vs resistant, Odds ratio (OR) =1.5, 95% confidence limits=0.9-2.5, χ^2 (Yates uncorrected)=3.0, p=0.08, GG = susceptibility to lung cancer

Table 14a. Cytochrome P450 2E1 1019 G/C Pst1 polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	C	G	CC	CG	GG
Lung Cancer n=109	10 (5%)	208 (95%)	0 (0%)	10[1] (9%)	99 (91%)
Resistant n=197 (%)	11 (3%)	383 (97%)	0 (0%)	11 (6%)	186 (94%)

* number of chromosomes (2n)

1. Genotype. CG vs GG for Lung cancer and resistant, Odds ratio (OR) =1.7, 95% confidence limits=0.7-4.5, χ^2 (Yates uncorrected)=1.42, p=0.23,
   CG = susceptibility to lung cancer

Table 14b. Cytochrome P450 2E1 C/T Rsa I polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	T	C	TT	TC	CC
Lung Cancer n=108	11 (5%)	205 (95%)	0 (0%)	11[1] (10%)	97 (90%)
Resistant n=198 (%)	11 (3%)	385 (97%)	0 (0%)	11 (6%)	187 (94%)

* number of chromosomes (2n)

1. Genotype. TC vs CC for Lung cancer and resistant, Odds ratio (OR) =1.9, 95% confidence limits=0.8-5.0, χ^2 (Yates uncorrected)=2.24, p=0.13,
   TC = susceptibility to lung cancer

Table 15a. Interleukin 18 105 A/C polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	C	A	CC	AC	AA
Lung Cancer n=107	50 (23%)	164 (77%)	8 (8%)	34 (33%)	65[1] (61%)
Resistant n=200 (%)	116 (29%)	284 (71%)	17[1] (9%)	82[1] (41%)	101 (50%)

* number of chromosomes (2n)

1. Genotype. AA vs AC/CC for Lung cancer and resistant, Odds ratio (OR) =1.6, 95% confidence limits=1.0-2.6, χ^2 (Yates uncorrected)=3.51, p=0.06,
   AA = susceptibility to lung cancer
   AC/CC= protective for lung cancer

Table 15b. Interleukin 18 -133 C/G polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*		Genotype		
	G	C	GG	CG	CC
Lung Cancer n=109	52 (24%)	166 (76%)	8 (7%)	36 (33%)	65[1] (60%)
Resistant n=198 (%)	117 (30%)	279 (70%)	17[1] (9%)	83[1] (42%)	98 (49%)

* number of chromosomes (2n)

1. Genotype. CC vs CG/GG for Lung cancer and resistant, Odds ratio (OR) =1.5, 95% confidence limits=0.9-2.5, χ^2 (Yates uncorrected)=2.90, p=0.09,
   CC = susceptibility to lung cancer

CG/GG = protective for lung cancer

Table 16. Glutathione S-Transferase M null polymorphism allele and genotype frequencies in the lung cancer subjects and resistant smokers.

Frequency	Allele*	
	Null	Wild
Controls n=178	75 (42%)	103 (58%)
Lung Cancer n=107	67[1] (58%)	48 (42%)
Resistant n=182	100 (55%)	82 (45%)

* number of chromosomes (2n)

1. Genotype. Null vs wild for Lung cancer and controls, Odds ratio (OR) =1.92, 95% confidence limits=1.2-3.2, χ^2 (Yates corrected)=6.64, p=0.01, Null = susceptibility to lung cancer

Table 17. Interferon-gamma 874 A/T polymorphism allele and genotype frequencies in the lung cancer subjects, 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%)
Lung cancer n=106 (%)	116 (55%)	96 (45%)	34[1, 2] (32%)	48 (45%)	24 (23%)
Resistant n=196 (%)	209 (53%)	183 (47%)	50 (26%)	109 (56%)	37 (19%)

* number of chromosomes (2n)

1. Genotype. AA vs AT/TT for lung cancer vs resistant, Odds ratio (OR) =1.4, 95% confidence limits 0.8-2.4, χ^2 (Yates uncorrected)= 1.48, p=0.22, AA genotype = susceptibile to lung cancer
2. Genotype. AA vs AT/TT for lung cancer vs controls, Odds ratio (OR) =1.9, 95% confidence limits 1.1-3.4, χ^2 (Yates corrected)= 5.45, p=0.02, AA genotype = susceptibile to lung cancer

Table 18. Cyclo-oxygenase 2 polymorphism allele and genotype frequency in the Lung cancer patients, exposed resistant smokers and controls.

Frequency	Allele*		Genotype		
	C	G	CC	CG	GG
Controls n=95 (%)	27 (14%)	161 (86%)	3 (3%)	21 (22%)	70 (75%)
Lung Cancer n=109 (%)	34 (16%)	184 (84%)	5 (5%)	24 (22%)	80 (73%)
Resistant n=158 (%)	75 (24%)	241 (76%)	11 (7%)	53 (34%)	94 (59%)

* number of chromosomes (2n)

1. Genotype. CC/CG vs GG for Lung cancer vs resistant, Odds ratio (OR) =0.53, 95% confidence limits=0.3-0.9, χ^2 (Yates corrected)= 4.9, p=0.03
   CC/CG =protective
2. Allele. C vs G for Lung cancer vs resistant, Odds ratio (OR) =0.59, 95% confidence limits 0.4- 0. 9, χ^2 (Yates corrected)= 4.8, p=0.03,
   C =protective
3. Genotype. GG vs CG/CC for Lung cancer vs resistant, Odds ratio (OR) =1.88, 95% confidence limits=1.1-3.3, χ^2 (Yates corrected)= 4.9, p=0.03
   GG =susceptibility
4. Allele. G vs C for Lung cancer vs resistant, Odds ratio (OR) =1.7, 95% confidence limits 1.1- 2.7, χ^2 (Yates corrected)= 4.8, p=0.03,
   G =susceptibility

Table 19. Matrix metalloproteinase 1 (MMP1) -1607 1G/2G polymorphism allele and genotype frequencies in Lung Cancer patients, exposed 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%)
Lung Cancer n=67 (%)	58 (43%)	76 (57%)	13 (19%)	32 (48%)	22 (33%)
Resistant n=171 (%)	167 (49%)	175 (51%)	41 (24%)	85 (50%)	45 (26%)

* number of chromosomes (2n)

1. Genotype. 2G2G vs 1G1G/1G2G for Lung cancer vs controls, Odds ratio (OR) =2.58, 95% confidence limits 1.3-5.2, χ^2 (Yates uncorrected)= 7.5, p=0.006
   2G2G genotype =susceptibility
2. Allele. 2G vs 1G for Lung cancer vs controls, Odds ratio (OR) =2.1, 95% confidence limits 1.4-3.2, χ^2 (Yates corrected)= 12.3, p=0.0004,
   2G = susceptibility

Table 20. Connective tissue growth factor (CTGF) -447 G/C polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	**Allele***		**Genotype**		
	G	C	GG	GC	CC
Lung cancer n=109 (%)	201 (92%)	17 (8%)	92 (84%)	17 (16%)	0 (0%)
Resistant n=200 (%)	379 (95%)	21 (5%)	179 (90%)	21 (10%)	0 (0%)

* number of chromosomes (2n)

1. Genotype. GC/CC vs GG for lung cancer vs resistant, Odds ratio (OR) =1.6, 95% confidence limits 0.8-3.3, χ^2 (Yates uncorrected)= 1.70, p=0.19, GC/CC genotype = susceptibility (trend)

Table 21. Mucin 5AC (Muc5AC) -221 C/T polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
Lung cancer n=109 (%)	177 (81%)	41 (19%)	73 (67%)	31 (28%)	5 (5%)
Resistant n=195 (%)	296 (76%)	94 (24%)	119 (61%)	58 (30%)	18 (9%)

* number of chromosomes (2n)

1. Genotype. TT vs CC/CT for lung cancer vs resistant, Odds ratio (OR) =0.47, 95% confidence limits 0.2-1.4, χ^2 (Yates uncorrected)= 2.16, p=0.14, TT genotype = protective (trend)

Table 22. Mannose binding lectin (MBL2) 161 G/A polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	G	A	GG	AG	AA
Lung cancer n=105 (%)	173 (82%)	37 (18%)	71 (67%)	31 (30%)	3 (3%)
Resistant n=197 (%)	338 (86%)	56 (14%)	147 (75%)	44 (22%)	6 (3%)

* number of chromosomes (2n)

1. Genotype. AG/AA vs GG for lung cancer vs resistant, Odds ratio (OR) =1.4, 95% confidence limits 0.8-2.4, χ^2 (Yates uncorrected)= 1.67, p=0.20, AG/AA genotype = susceptibility (trend)

Table 23. Nibrin (NBS1) Gln185Glu G/C polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	G	C	GG	GC	CC
Lung cancer n=109 (%)	150 (69%)	68 (31%)	54 (50%)	42 (39%)	13 (12%)
Resistant n=199 (%)	295 (74%)	103 (26%)	107 (54%)	81 (41%)	11 (6%)

* number of chromosomes (2n)

1. Genotype. CC vs CG/GG for lung cancer vs resistant, Odds ratio (OR) =2.3, 95% confidence limits 0.9-5.8, χ^2 (Yates uncorrected)= 4.01, p=0.05, CC genotype = susceptibility

Table 24. Arginase 1 (Arg1) intron 1 C/T polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
Lung cancer n=105 (%)	137 (65%)	73 (35%)	45 (43%)	47 (45%)	13 (12%)
Resistant n=180 (%)	203 (56%)	157 (44%)	65 (36%)	73 (41%)	42 (23%)

* number of chromosomes (2n)

1. Genotype. TT vs CC/CT for lung cancer vs resistant, Odds ratio (OR) =0.46, 95% confidence limits 0.2-0.95, χ^2 (Yates uncorrected)= 5.11, p=0.02, TT genotype = protective
2. Allele. T vs C for lung cancer vs resistant, Odds ratio (OR) =0.69, 95% confidence limits 0.5-1.0, χ^2 (Yates corrected)= 3.96, p=0.05, T allele = protective

Table 25. REV1 Phe257Ser C/T polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	C	T	CC	CT	TT
Lung cancer n=109 (%)	129 (59%)	89 (41%)	39 (36%)	51 (47%)	19 (17%)
Resistant n=192 (%)	242 (63%)	142 (37%)	83 (43%)	76 (40%)	33 (17%)

* number of chromosomes (2n)

1. Genotype. CC vs CT/TT for lung cancer vs resistant, Odds ratio (OR) =0.73, 95% confidence limits 0.4-1.2, χ^2 (Yates uncorrected)= 1.6, p=0.20, CC genotype = protective (trend)

Table 26. Insulin-like growth factor II receptor (IGF2R) Leu252Val C/G polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	C	G	CC	CG	GG
Lung cancer n=109 (%)	190 (87%)	28 (13%)	82 (75%)	26 (24%)	1 (1%)
Resistant n=198 (%)	342 (86%)	54 (14%)	150 (76%)	42 (21%)	6 (3%)

* number of chromosomes (2n)

1. Genotype. GG vs CC/CG for lung cancer vs resistant, Odds ratio (OR) =0.30, 95% confidence limits 0.01-2.5, χ^2 (Yates uncorrected)= 1.41, p=0.22 (1-tailed t-test), GG genotype = protective (trend)

Table 27. Apex nuclease (APE1) Asp148Glu T/G polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	T	G	TT	TG	GG
Lung cancer n=109 (%)	124 (57%)	94 (43%)	39 (36%)	46 (42%)	24 (22%)
Resistant n=192 (%)	229 (60%)	155 (40%)	69 (36%)	91 (47%)	32 (17%)

* number of chromosomes (2n)

1. Genotype. GG vs TT/TG for lung cancer vs resistant, Odds ratio (OR) =1.4, 95% confidence limits 0.8-2.7, χ^2 (Yates uncorrected)= 1.3, p=0.25, GG genotype = susceptibility (trend)

Table 28. Interleukin 10 (IL-10) -1082 A/G polymorphism allele and genotype frequencies in the lung cancer and resistant smokers.

Frequency	Allele*		Genotype		
	G	C	GG	GC	CC
Lung cancer n=98 (%)	91 (46%)	105 (54%)	16 (16%)	59 (60%)	23 (24%)
Resistant n=196 (%)	174 (44%)	218 (56%)	40 (20%)	94 (48%)	62 (32%)

* number of chromosomes (2n)

1. Genotype. GG vs GC/CC for lung cancer vs resistant, Odds ratio (OR) =0.66, 95% confidence limits 0.4-1.2, χ^2 (Yates uncorrected)= 2.12, p=0.15, GG genotype = protective (trend)

Table 29. Summary of protective and susceptibility polymorphisms in Lung Cancer patients relative to resistant smokers (with normal lung function)

Gene	Polymorphism	Role
Nitric Oxide synthase 3 (NOS3)	NOS3 Asp 298 Glu	TT protective
Nitric Oxide synthase 3 (NOS3)	NOS3 -786 T/C	TT susceptibility
Superoxide dismutase 3 (SOD3)	SOD3 Arg 312 Gln	CG/GG protective
XRCC1	XRCC1 Arg 399 Gln G/A	AA protective
Interleukin-8 (IL-8)	IL-8 -251 A/T	AA protective
Anti-chymotrypsin (ACT)	ACT Ala 15 Thr	GG susceptibility
Cyclin D (CCND1)	CCND1 A870G	GG protective AA susceptibility
Interleukin 1B (IL-1B)	IL-1B -511 A/G	GG susceptibility
FAS (Apo-1/CD95)	FAS A-670G	AA susceptibility
XPD	XPD -751 G/T	GG protective
CYP 1A1	CYP 1A1 Ile 462 Val A/G	GG/AG protective AA susceptibility
Matrix metalloproteinase 12 (MMP12)	MMP12 Asn 357 Ser A/G	GG/AG protective
8-Oxoguanine DNA glycolase (OGG1)	OGG1 Ser 326 Cys G/C	GG protective
N-acetyltransferase 2 (NAT2)	NAT2 Arg 197 Gln A/G	GG susceptibility
CYP2E1	CYP2E1 1019 G/C Pst I	CC/CG susceptibility
CYP2E1	CYP2E1 C/T Rsa I	TT/TC susceptibility

Interleukin -18 (IL-18)	IL-18 105 A/C	AC/CC protective AA susceptibility
Interleukin -18 (IL-18)	IL-18 -133 G/C	CG/GG protective CC susceptibility
Glutathione S-transferase M	GSTM null	Null susceptibility
Interferon gamma (IFNγ)	IFNγ 874 A/T	AA susceptibility
Cyclo-oxygenase 2 (COX2)	COX2 -765 G/C	CC/CG protective GG susceptibility
Matrix metalloproteinase 1 (MMP1)	MMP -1607 1G/2G	2G2G susceptibility
Connective tissue growth factor (CTGF)	CTGF -447 G/C	GC/CC susceptibility
Mucin 5AC (MUC5AC)	MUC5AC -221 C/T	TT protective
Mannose binding lectin 2 (MBL2)	MBL2 +161 G/A	AG/AA susceptibility
Nibrin (NBS1)	NBS1 Gln185Glu G/C	CC susceptibility
Arginase 1 (Arg1)	Arg1 intron 1 C/T	TT protective
REV1	REV1 Phe257Ser C/T	CC protective
Insulin-like growth factor II receptor (IGF2R)	IGF2R Leu252Val C/G	GG protective
Apex nuclease (Apex or APE1))	Apex Asp148Glu G/T	GG susceptibility
Interleukin 10 (IL-10)	IL-10 -1082 A/G	GG protective

Table 30. Combined frequencies of the presence or absence of protective genotypes (CYP1A1 GG/AG, OGG1 GG, CCND1 GG, NOS3 298 TT, IL-8 AA, XRCC1 AA) **in the exposed smoking subjects (Lung cancer subjects and resistant smokers) .**

	Number of protective polymorphisms			
Cohorts	0	1	≥2	Total
Lung Cancer	66 (61%)	37 (34%)	6 (6%)	109
Resistant smokers	71 (36%)	86 (43%)	42 (21%)	199
% of smokers with Lung cancer	66/137 (48%)	37/123 (30%)	6/42 (14%)	

Comparison	Odd's ratio	95% CI	χ^2	P value
0 vs 1 vs 2+, Resist vs Lung cancer	-	-	22.3	<0.0001
2+ vs 0-1, Resist vs Lung cancer	4.6	1.8-12.5	11.87	0.0005
0 vs 2+, Lung cancer vs Resist	2.8	1.7-4.6	16.7	<0.0001

Table 31. Combined frequencies of the presence or absence of susceptibility genotypes (CYP2E1 1019 CC/CG, FAS AA, IL-1B GG, ACT 15 GG) **in the exposed smoking subjects (Lung cancer subjects and resistant smokers).**

	Number of susceptibility polymorphisms			
Cohorts	0	1	≥2	Total
Lung Cancer	21 (19%)	52 (48%)	35 (33%)	108
Resistant smokers	71 (36%)	85 (43%)	42 (21%)	198
% of smokers with lung cancer	21/92 (23%)	52/137 (38%)	35/77 (45%)	

Comparison	Odd's ratio	95% CI	χ^2	P value
0 vs 1 vs 2+, Lung cancer vs Resist	-	-	10.2	0.006
2+ vs 0-1, Lung cancer vs Resist	1.8	1.0-3.1	4.1	0.04

0+ vs 1-2+, Resist vs Lung cancer	2.3	1.3-4.2	8.2	0.004

Table 32. Combined frequencies of the presence or absence of protective genotypes (CYP1A1 GG/AG, OGG1 GG, CCND1 GG, NOS3 298 TT, SOD3 CG/GG, XPD GG, MMP12 GG/AG, XRCC1 AA, n=8) **in the exposed smoking subjects (Lung cancer subjects and resistant smokers) .**

	Number of protective polymorphisms n=8			
Cohorts	0	1	≥2	Total
Lung Cancer	54 (50%)	50 (46%)	5 (4%)	109
Resistant smokers	67 (34%)	83 (42%)	50 (25%)	199
% of smokers with Lung cancer	54/121 (45%)	50/133 (38%)	5/55 (9%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, Resist vs Lung cancer	-	-	21.5	<0.0001
2+ vs 0-1, Resist vs Lung cancer	6.9	2.5-20.5	18.7	<0.0001
0 vs 2+, Lung cancer vs Resist	2.0	1.2-3.2	6.96	0.008

Table 33. Combined frequencies of the presence or absence of susceptibility genotypes (CYP2E1 1019 CC/CG, FAS AA, IL-1B GG, ACT 15 GG, NAT2 GG, IL-18 105 AA, IFNγ AA, n=7) **in the exposed smoking subjects (Lung cancer subjects and resistant smokers).**

	Number of susceptibility polymorphisms n=7			
Cohorts	1	2	≥3	Total
Lung Cancer	16 (15%)	35 (32%)	58 (53%)	109
Resistant smokers	65 (33%)	66 (33%)	69 (34%)	200
% of smokers with Lung cancer	16/81 (20%)	35/101(35%)	58/127(46%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, Lung cancer vs Resist	-	-	14.6	0.0007
3+ vs 1-2, Lung cancer vs Resist	2.2	1.3-5.6	9.4	0.002
1 vs 2-3+, Resist vs Lung cancer	2.8	1.5-5.4	10.7	0.001

Table 34. Combined frequencies of the presence or absence of protective genotypes (CYP1A1 GG/AG, OGG1 GG, CCND1 GG, NOS3 298 TT, IL-8 AA, XRCC1 AA, Cox 2 -765 CC/CG) **in the exposed smoking subjects (Lung cancer subjects and resistant smokers).**

	Number of protective polymorphisms			
Cohorts	0	1	≥2	Total
Lung Cancer	45 (40%)	50 (43%)	19 (17%)	114
Resistant smokers	47 (23%)	79 (40%)	74 (37%)	200

% of smokers with Lung cancer	45/47 (49%)	50/129 (39%)	19/93 (20%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, Resist vs Lung cancer	-	-	16.8	0.0002
2+ vs 0-1, Resist vs Lung cancer	2.94	1.6-5.4	13.44	0.0002
0 vs 2+, Lung cancer vs Resist	2.12	1.3-3.6	8.2	0.004

Table 35. Combined frequencies of the presence or absence of susceptibility genotypes (CYP2E1 1019 CC/CG, FAS AA, IL-B1 GG, ACT 15 GG, MMP1 2G2G) **in the exposed smoking subjects (Lung cancer subjects and resistant smokers).**

	Number of suceptibility polymorphisms			
Cohorts	0-1	2-3	4-6	Total
Lung Cancer	13 (12%)	66 (61%)	30 (28%)	109
Resistant smokers	54 (27%)	113 (56%)	33 (17%)	200
% of smokers with Lung cancer	13/67 (19%)	66/179 (37%)	30/63 (48%)	

Comparison	Odd's ratio	95% CI	$\chi 2$	P value
0 vs 1 vs 2+, Lung cancer vs Resist	-	-	11.8	0.003
2+ vs 0-1, Lung cancer vs Resist	1.9	1.0-3.5	4.6	0.03
0+ vs 1-2+, Resist vs Lung cancer	2.7	1.4-5.6	8.6	0.003

DISCUSSION

The above results show that several polymorphisms were associated with either increased or decreased risk of developing lung cancer. 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 subjects from those who are resistant (for example, between the smokers who develop lung cancer and those with the least risk with comparable smoking exposure). 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 a number of genes involved in processes known to underlie lung remodelling and lung cancer. The polymorphisms identified here are found in genes encoding proteins central to these processes which include inflammation, matrix remodelling, oxidant stress, DNA repair, cell replication and apoptosis.

In the comparison of smokers with lung cancer and matched smokers with near normal lung function (lowest risk for lung cancer despite smoking), several polymorphisms were identified as being found in significantly greater or lesser

frequency than in the comparator groups (sometimes including the blood donor cohort). Due to the small cohort of lung cancer patients, polymorphisms where there are only trends towards differences (P=0.06-0.25) were included in the analyses, although in the combined analyses only those polymorphisms with the most significant differences were utilised.

- In the analysis of the Asp 298 Glu (T/G) polymorphism of the Nitric oxide synthase 3 gene, the TT genotype was found to be greater in the smoking resistant cohort compared to the lung cancer cohort (OR=1.8, P=0.14) consistent with a protective role. This greater frequency compared to the blood donor cohort also suggests that the TT genotype is over represented in the resistant group (see Table 1a).
- In the analysis of the -786 T/C polymorphism of the Nitric oxide synthase 3 gene, the TT genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.4, P=0.23) consistent with a susceptibility role (see Table 1b).
- In the analysis of the Arg 312 Gln polymorphism of the Superoxide dismutase 3 gene, the CG and GG genotypes were found to be significantly greater in the smoking resistant cohort compared to the lung cancer cohort (P=0.03) consistent with each having a protective role (Table 2).
- In the analysis of the Arg 399 Gln A/G polymorphism of the XRCC1 gene, the AA genotype was found to be greater in the smoking resistant cohort compared to the lung cancer cohort (OR=2.6, P=0.09) consistent with a protective role (Table 3).
- In the analysis of the -251 A/T polymorphism of Interleukin-8 gene, the AA genotype was found to be significantly greater in the smoking resistant cohort compared to the lung cancer cohort (OR=4.1, P=0.002) consistent with a protective role (Table 4). The A allele was also found to be significantly greater in the smoking resistant cohort compared to the lung cancer cohort (OR = 1.5, P = 0.02) consistent with a protective role (Table 4).
- In the analysis of the Ala 15 Thr polymorphism of Anti-chymotrypsin gene, the GG genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.7, P=0.06) consistent with a susceptibility role (see Table 5). The G allele was found to be greater in the lung cancer cohort

compared to the smoking resistant cohort (OR = 1.3, P = 0.1) consistent with a susceptibility role (see Table 5).

- In the analysis of the A 870 G polymorphism of the Cyclin D1 gene, the GG genotype was found to be greater in the smoking resistant cohort compared to the lung cancer cohort (OR=1.4, P=0.20) consistent with a protective role (see Table 6). In contrast, the AA genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR = 1.4, P = 0.2) consistent with a susceptibility role (see Table 6).
- In the analysis of the -511 A/G polymorphism of the Interleukin 1B gene, the GG genotype was found to be significantly greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.6, P=0.04) consistent with a susceptibility role (see Table 7).
- In the analysis of the A -670 G polymorphism of the FAS (Apo-1/CD95) gene, the AA genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.5, P=0.15) consistent with a susceptibility role (see Table 8). The A allele was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR = 1.3, P = 0.15) consistent with a susceptibility role (see Table 8).
- In the analysis of the 751 T/G polymorphism of the XPD gene, the GG genotype was found to be greater in the smoking resistant cohort compared to the lung cancer cohort (OR=1.7, P=0.18) consistent with a protective role (Table 9).
- In the analysis of the Ile 462 Val G/A polymorphism of the CYP 450 1A1 gene, the AG and GG genotypes were found to be greater in the smoking resistant cohort compared to the lung cancer cohort (OR=2.2, P=0.12) consistent with each having a protective role (see Table 10). In contrast, the AA genotype was found to be consistent with a susceptibility role (see Table 10).
- In the analysis of the Asn 357 Ser polymorphism of the Matrix metalloproteinase 12 gene, the GG and AG genotypes were found to be greater in the smoking resistant cohort compared to the lung cancer cohort (OR=1.7, P=0.23) consistent with each having a protective role (Table 11).
- In the analysis of the Ser 326 Cys (C/G) polymorphism of the OGG1 gene, the GG genotype was found to be significantly greater in the smoking resistant

cohort compared to the lung cancer cohort (OR=4.0, P=0.05) consistent with a protective role (Table 12).

- In the analysis of the Arg 197 Gln G/A polymorphism of the N-Acetyltransferase 2 gene, the GG genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.5, P=0.08) consistent with a susceptibility role (see Table 13).
- In the analysis of the 1019 G/C Pst 1 polymorphism of the CYP 2E1 gene, the CG genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.7, P=0.23) consistent with a susceptibility role (see Table 14a).
- In the analysis of the C/T Rsa 1 polymorphism of the CYP 2E1 gene, the TC genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.9, P=0.13) consistent with a susceptibility role (see Table 14b).
- In the analysis of the 105 A/C polymorphism of the Interleukin-18 gene, the AA genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.6, P=0.06) consistent with a susceptibility role (see Table 15a). In contrast, the AC and CC genotypes were each consistent with a protective role (see Table 15).
- In the analysis of the -133 C/G polymorphism of the Interleukin-18 gene, the CC genotype was found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR=1.5, P=0.09) consistent with a susceptibility role (see Table 15b). In contrast, the CG and GG genotypes were each consistent with a protective role (see Table 15b).
- In the analysis of the null polymorphism of the Glutathione S Transferase gene, the null genotype was found to be greater in the lung cancer cohort compared to the blood donor cohort (OR=1.92, P=0.01) consistent with a susceptibility role (see Table 16).
- In the analysis of the 874 A/T polymorphism of the Interferon gamma gene, the AA genotype was found to be greater in the lung cancer cohort compared to the blood donor cohort (OR=1.9, P=0.02) and compared to the smoking resistant cohort (OR = 1.4, P = 0.22) consistent with a susceptibility role (see Table 17).

- In the analysis of the -765 C/G promoter polymorphisms of the cyclo-oxygenase 2 gene, the C allele, and CCand CG genotypes were found to be significantly greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.59, P = 0.03 and OR = 0.53, P = 0.03, respectively), consistent with a protective role. This 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 18). In contrast, the G allele and GG genotype were found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR = 1.7, P = 0.03 and OR = 1.88, P = 0.03, respectively), consistent with a susceptibility role (see Table 18).
- In the analysis of the -1607 1G/2G polymorphisms of the Matrix metalloproteteinase 1 gene, the 2G2G genotype was found to be significantly greater in the lung cancer cohort compared to the blood donor cohort (OR = 2.58, P = 0.006), consistent with a susceptibility role (see Table 19). The 2G allele was found to be significantly greater than the lung cancer cohort compared to the blood donor cohort (OR = 2.1, P = 0.0004), consistent with a susceptibility role (see Table 19).
- In the analysis of the -447 G/C polymorphism of the connective tissue growth factor gene, the CC and GC genotypes were found to be greater in the lung cancer cohort compared to the smoking resistant cohort (OR = 1.6, P = 0.19) consistent with a susceptibility role (see Table 20).
- In the analysis of the -221 C/T polymorphism in the Mucin 5AC gene the TT genotype was found to be greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.47, P = 0.14) consistent with a protective role (see Table 21).
- In the analysis of the 161 G/A polymorphism of the Mannose binding lectin (MBL2) gene, the AA and AG genotypes were found to be greater in the lung cancer cohort compared to the resistant smoker cohort (OR = 1.4, P = 0.20) consistent with each having a susceptibility role (see Table 22).
- In the analysis of the Gln 185 Glu G/C polymorphism of the Nibrin gene, the CC genotype was found to be significantly greater in the lung cancer cohort compared to the resistant smoker cohort (OR = 2.3, P = 0.05), consistent with a susceptibility role (see Table 23).

- In the analysis of the intron 1 C/T polymorphism of the Arginase 1 gene, the TT genotype was found to be significantly greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.46, P = 0.02) consistent with a protective role (see Table 24). The T allele was found to be significantly greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.69, P = 0.05) consistent with a protective role (see Table 24).
- In the analysis of the Phe 257 Ser C/T polymorphism of the REV1 gene, the CC genotype was found to be greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.73, P = 0.20), consistent with a protective role (see Table 25).
- In the analysis of the Leu 252 Val C/G polymorphism of the Insulin-like growth factor II receptor gene the GG genotype was found to be greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.30, P = 0.22) consistent with a protective role (see Table 26).
- In the analysis of the Asp 148 Glu T/G polymorphism of the Apex nuclease gene, the GG genotype was found to be greater in the lung cancer cohort compared to the resistant smoker cohort (OR = 1.4, P = 0.25), consistent with a susceptibility role (see Table 27).
- In the analysis of the -108 2A/G polymorphism of the Interleukin-10 gene the GG genotype was found to be greater in the resistant smoker cohort compared to the lung cancer cohort (OR = 0.66, P = 0.15), consistent with a protective role (see Table 28).

It is accepted that the disposition to lung cancer is the result of the combined effects of the individual's genetic makeup and other factors, including their lifetime exposure to various aero-pollutants including tobacco smoke. Similarly it is accepted that lung cancer 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 lung cancer. A number of genetic mutations working in combination either promoting or protecting the lungs from damage are likely to be involved in elevated resistance or susceptibility to lung cancer.

From the analyses of the individual polymorphisms, 17 protective genotypes were identified and analysed for their frequencies in the smoker cohort consisting of low risk smokers, i.e., resistant smokers (near normal lung function) and those with lung cancer.

When the frequencies of resistant smokers and smokers with lung cancer were compared according to the presence of 0, 1 and 2+ protective genotypes selected from a subset of six of the protective genotypes (CYP1A1 GG/AG, OGG1 GG, CCND1 GG, NOS3 298 TT, IL-8 AA, XRCC1 AA), significant differences were found (overall $\gamma 2=22.3$, $P<0.0001$) (see Table 30). This analysis suggests smokers with 2+ protective genotypes had 4-5 times more likelihood of being resistant (OR=4.6, P=0.0005), while those with no protective genotypes were nearly three times as likely to have lung cancer (OR=2.8, $P<0.0001$). Examined another way, the chance of having lung cancer diminished from 48%, to 30%, to 14%, in smokers with 0, 1, or 2+ protective genotypes, respectively. Analyses of larger groups of protective polymorphisms resulted in similar findings (see Tables 32 and 34).

From the analyses of the individual polymorphisms, 19 possible susceptibility genotypes were identified and analysed for their frequencies in the smoker cohort consisting of resistant smokers and those with lung cancer. When the frequencies of resistant smokers and smokers with lung cancer were compared according to the presence of 0, 1 and 2+ susceptibility genotypes selected from a subset of six of the susceptibility genotypes (CYP2E1 1019 CC/CG, FAS AA, IL-1B GG, ACT 15 GG genotypes) significant differences were found (overall $\gamma 2=10.2$, P=0.006) (see Table 31). This analysis suggests that smokers with 2+ susceptibility genotypes had nearly two times more likelihood of having lung cancer (OR=1.8, P=0.04), while those with no susceptibility genotypes were two fold as likely to be resistant (OR=2.3, P=0.004). Examined another way, the chances of having lung cancer increased from 23%, to 38%, to 45% in smokers with 0, 1 or 2+ susceptibility genotypes, respectively. Analyses of larger groups of susceptibility polymorphisms resulted in similar findings (see Tables 33 and 35).

These findings indicate that the methods of the present invention may be predictive of lung cancer 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 risk of developing lung

cancer, 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, and/or additional therapeutic approaches such as gene therapy or RNAi. In another example, as shown herein the -133 C allele in the promoter of the gene encoding IL-18 is associated with susceptibility to lung cancer. The -133 G allele in the promoter of the gene encoding IL-18 is associated with increased IL-18 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 IL-18. An alternative therapy may be to administer IL-18 or an functional analogue thereof to a subject or to otherwise augment IL-18 levels in the subject. In a further example, as shown herein the -1607 2G/2G genotype in the promoter of the gene encoding MMP1 is associated with susceptibility to lung cancer. A number of inhibitors of matrix metalloproteinases are known, for example those discussed in US 6, 600,057 (incorporated herein in its entirety), such as tissue inhibitors of metalloproteinases (TIMPs) including TIMP1, TIMP2, TIMP3, and TIMP4, which form inactive complexes with MMPs, more general proteinase regulators which prevent MMP action, regulators of MMP gene expression including membrane bound MMPs (MT-MMP) that activate the excreted proenzyme form of MMPs, and compounds such as 4,5-dihydroxyanthaquinone-2-carboxylic acid (AQCA) and derivatives thereof. A suitable therapy in subjects known to possess the -1607 2G/2G genotype can be the administration of an agent capable of reducing expression of the gene encoding MMP1, or administration of an agent capable of reducing the activity of MMP1, for example by administration of an agent capable of increasing expression of or the activity of one or more TIMPs, or administration of an agent capable of reducing expression of or the activity of one or more membrane bound MMPs or other activators of MMP1. For example, a suitable therapy can be the administration to such a subject of a MMP1 inhibitor such as 4,5-dihydroxyanthaquinone-2-carboxylic acid (AQCA), anthraquinyl-mercaptoethyamine, anthraquinyl-alanine hydroxamate, or derivatives thereof. In another example, a given susceptibility genotype is associated with increased expression of a gene relative to that observed with the protective genotype. A suitable therapy in subjects known to possess the susceptibility genotype is the administration of an agent capable of reducing expression of the gene, for example using antisense or RNAi methods. An alternative suitable therapy can be the administration to such a subject of

an inhibitor of the gene product. In still another example, a susceptibility genotype present in the promoter of a gene is associated with increased binding of a repressor protein and decreased transcription of the gene. A suitable therapy is 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 gene having a reduced affinity for repressor binding (for example, a gene copy having a protective 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.

EXAMPLE 2

Table 36 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 parentheses. The rs numbers provided are identifiers unique to each polymorphism.

Table 36. Polymorphism reported to be in LD with polymorphisms specified herein.

NOS3

rs2373962	rs3918225	rs3918169	rs2373961	rs3918160
rs3918170	rs6951150	rs1800779	rs3793342	rs13238512
rs2243311	rs3793341	rs10247107	rs3918161	rs1549758
rs10276930	rs10952298	rs1007311	rs10277237	rs2070744 **(-786 T/C)**
rs9282803	rs12703107	rs9282804	rs6946340	rs3918226
rs1799983 **(Asp 298 Glu)**	rs6946091	rs3918162	rs6946415	rs3918163
rs10952296	rs3918164	rs13309715	rs3918165	rs10952297
rs1800781	rs7784943	rs13310854	rs11771443	rs13310763
rs2243310	rs2853797	rs1800783	rs13311166	rs3918155
rs13310774	rs3918156	rs2853798	rs2566519	rs11974098
rs3918157	rs3918166	rs3918158	rs3730001	rs3918159
rs3918167	rs2566516	rs3918168		

XRCC1

rs1799782	rs2307175	rs2307173	rs2307192	rs2307176
rs25492	rs6413430	rs3213363	rs25493	rs909008
rs3213365	rs25485	rs1799780	rs25488	rs3213367
rs3213360	rs25489	rs25486	rs3213361	rs2307188
rs2271980	rs915927	rs3213366	rs25487 **(Arg 399 Gln)**	rs3192714
rs25490	rs10407677	rs25491		

IL8

rs4694635	rs2227527	rs2227543	rs11730560	rs11730284
rs1957663	rs7682639	rs12420	rs13106097	rs11944402
rs4694636	rs2227529	rs16849942	rs4694178	rs7658422
rs16849925	rs2227530	rs3181685	rs4694637	rs11940656
rs16849928	rs2227531	rs11733933	rs11729759	rs1951700
rs11730667	rs2227532	rs2227544	rs10938093	rs1951699
rs16849934	rs2227534	rs2227545	rs13109377	rs1957662
rs4073 **(-251 A/T)**	rs2227550	rs1951236	rs16849938	rs2227546
rs1951237	rs6831816	rs2227535	rs1126647	rs6446955
rs2227517	rs2227536	rs11545234	rs6446956	rs2227518
rs2227537	rs2227548	rs6446957	rs2227519	rs2227538
rs10938092	rs16849945	rs2227520	rs1803205	rs13112910
rs1951239	rs2227521	rs2227539	rs13142454	rs1951240
rs2227522	rs3756069	rs11937527	rs1957661	rs2227523
rs2227307	rs12647924	rs7674884	rs2227524	rs2227549
rs13152254	rs16849958	rs2227525	rs2227540	rs13138765
rs17202249	rs2227526	rs2227306	rs13139170	rs1951242

Anti-chymotrypsin, ACT

rs4900239	rs6575448	rs9323909	rs4905225	rs4362321
rs2896288	rs10131646	rs1884082	rs11160196	rs10131818
rs7492561	rs10131754	rs10133663	rs11845108	rs12886656
rs1004761	rs12886657	rs9671421	rs7493944	rs7151480
rs7493955	rs9671431	rs4934 **(ACT Ala 15 Thr)**	rs12433006	rs9671948
rs1800463	rs10135321	rs17826465	rs10150184	rs11538071
rs10150491	rs11538070			

CCND1

rs3862792	rs11557712	rs11604847	rs12049899	rs603965 **(A 870 G)**
rs678653	rs1051357	rs12288719	rs3212893	rs1803191
rs12283700	rs3918298	rs2510607	rs1803190	rs948886
rs3918299	rs3212894	rs3212910	rs948887	rs3918301

rs3212895	rs3212911	rs3212880	rs3212896	rs3212912
rs3212881	rs3212897	rs3212913	rs3212882	rs3212898
rs3212915	rs3212883	rs11557584	rs3212916	rs3212884
rs2062445	rs3212917	rs3781611	rs3212899	rs3212918
rs649392	rs3212900	rs3212919	rs3212885	rs3212901
rs12805391	rs3212886	rs3212902	rs3212920	rs3212887
rs3212903	rs3212921	rs3918302	rs3212904	rs3212922
rs7944853	rs1051062	rs11603541	rs3212888	rs3212905
rs7116781	rs3212889	rs3212906	rs7121246	rs3212890
rs3212907	rs7124951	rs3212891	rs3212908	rs12281701
rs2510467	rs3212909	rs12288567	rs3212892	rs3802782
rs1192925	rs7177	rs7178	rs1192926	

Interleukin 1B, IL1B

rs10169916	rs6743326	rs13009179	rs6743322	rs4849127
rs6761220	rs4849126	rs6761218	rs7558108	rs5021469
rs13032029	rs6710598	rs13013349	rs1143623	rs12623093
rs1143624	rs3087255	rs2708920	rs3087256	rs1143625
rs6721954	rs2853545	rs12621220	rs2708921	rs7596849
rs1143626	rs4848306	rs3087258	rs3087257	rs16944 **(C -511 T)**
rs7556811	rs7556903	rs3917346	rs6743438	rs4986962
rs6743427	rs1143627	rs6761336	rs6761335	rs6743338
rs6761245	rs6761237	rs6743330		

FAS, TNFRSF6

rs3758483	rs6586166	rs9658718	rs3218618	rs2234767
rs6586167	rs1926193	rs12720435	rs9658674	rs9658691
rs1926192	rs7910435	rs9658675	rs9658692	rs1926191
rs17114661	rs2234768	rs9658693	rs4244983	rs3218614
rs1800682 **(A -670 G)**	rs9658694	rs9658719	rs9658754	rs9658695
rs9658721	rs12720436	rs9658676	rs4345878	rs9658722
rs7911226	rs5030765	rs9658696	rs9658723	rs1926190
rs7474952	rs9658697	rs11202924	rs9658755	rs9658677
rs9658698	rs9658724	rs9658756	rs12251390	rs9658699
rs9658725	rs9658757	rs2274355	rs9658700	rs9658726
rs3781202	rs5030766	rs9658702	rs2296604	rs9658759
rs12775501	rs7094676	rs9658727	rs9658761	rs7079111
rs9658703	rs9658728	rs982764	rs3740286	rs9658704
rs9658729	rs9658763	rs4064	rs9658705	rs7069750
rs3218620	rs1324551	rs9658706	rs9658730	rs2296600
rs10509561	rs9658707	rs9658731	rs6586163	rs1571011
rs3218619	rs6586164	rs9658708	rs9333296	rs3824730
rs1571012	rs3218621	rs3781204	rs1571013	rs3218613
rs12571917	rs9658710	rs9658732	rs3824729	rs9658711
rs2296603	rs10887877	rs7911752	rs9658733	rs11596616
rs11591675	rs9658734	rs7076197	rs11591676	rs9658735
rs12359362	rs12766185	rs9658736	rs7097467	rs11591677
rs9658738	rs7097572	rs9658712	rs9658739	rs9658679
rs9658713	rs9658740	rs9658680	rs2147421	rs9658741
rs1926196	rs2147420	rs7896789	rs1926195	rs9658714
rs9658742	rs9658681	rs1159120	rs9658744	rs9325603
rs4406737	rs9658745	rs2031610	rs2148287	rs9658748
rs7897395	rs9658715	rs9658749	rs7909414	rs9658716
rs7901656	rs9658682	rs2147419	rs9658750	rs7069061
rs2148286	rs9658751	rs7072828	rs2147418	rs2031613
rs6586165	rs9658717	rs2031612	rs9658683	rs4406738
rs9658753	rs9658684	rs7916814	rs2296602	rs9658685
rs2182408	rs2031611	rs7913007	rs7920305	rs3218612

rs9658687	rs1926194	rs2296601		

XPD, ERCC2

rs1799793	rs238409	rs3916858	rs3916876	rs7257638
rs3916838	rs106433	rs238417	rs3916816	rs50871
rs3916860	rs3916878	rs3916817	rs50872	rs3916861
rs3916879	rs3916818	rs3916839	rs3916862	rs1799787
rs3916819	rs3916840	rs3916863	rs1799788	rs3916820
rs3916841	rs238412	rs1799789	rs238404	rs3916842
rs3916864	rs16979773	rs3916821	rs3916843	rs11668936
rs1052555	rs3916822	rs3916844	rs3916866	rs3916881
rs238403	rs7251321	rs2070831	rs3916882	rs171140
rs3916845	rs3916868	rs3916883	rs3895625	rs3916846
rs238413	rs238418	rs3916824	rs3916847	rs238414
rs3916885	rs3916825	rs3916848	rs3916870	rs3916886
rs3916826	rs238410	rs3916871	rs1799790	rs3916827
rs238411	rs3916872	rs13181 **(751 G/T)**	rs3916830	rs3916849
rs238415	rs3916831	rs3916850	rs3916873	rs3916832
rs3916851	rs3932979	rs3916833	rs3916853	rs238416
rs3916834	rs3916854	rs3916874	rs3916835	rs3916855
rs11667568	rs3916836	rs3916856	rs3916875	rs3916837
rs3916857	rs11666730			

CYP1A1

rs11631784	rs4646418	rs2515900	rs7496395	rs2856844
rs4646903	rs7496533	rs2606345	rs2472309	rs4886605
rs8031941	rs2472308	rs12441817	rs4646420	rs12915975
rs2470891	rs4646421	rs6495121	rs2470892	rs4986885
rs1456432	rs7182554	rs2606344	rs2198843	rs936225
rs4646422	rs11072499	rs7180012	rs2229150	rs8039800
rs4986879	rs7179952	rs4987133	rs7181062	rs2856833
rs7179590	rs1799814	rs7180066	rs1048943 **(Ile 462 Val)**	rs7495708
rs2470893	rs2278970	rs3809585	rs2606346	rs2445619
rs4986880	rs2472296	rs4986881	rs4646417	rs4986882
rs2856831	rs1800031	rs2856832	rs4986883	rs3826042
rs4986884	rs3826041	rs2472307		

MMP12

rs652438 **(Asn 357 Ser)**	rs641519	rs585007	rs640735	rs672745
rs651159	rs644008	rs672743	rs644552	rs1291647
rs12794039	rs476185	rs1291646	rs12796315	rs476391
rs1295870	rs505770	rs1291645	rs1042509	rs597518
rs626729	rs626407	rs686375	rs626393	rs12808148
rs1291643	rs484171	rs1296235	rs660727	rs1291642
rs7102181	rs1296234	rs674546	rs1794204	rs7128711
rs580266	rs660599	rs615932	rs511081	rs737693
rs654600	rs686439	rs645763	rs518509	rs561849
rs586701	rs641957	rs11821430	rs1296237	rs1299505
rs641920	rs673217	rs644885	rs673163	

OGG1

rs159154	rs11548133	rs3219007	rs2471902	rs17291491
rs3219001	rs3219008	rs2308329	rs12498111	rs17050550
rs3219009	rs2072668	rs454071	rs1801127	rs159150
rs3219012	rs11928210	rs2472031	rs1805373	rs2471903
rs17050542	rs3219002	rs2472038	rs2472042	rs17775374
rs2471894	rs3219010	rs3219013	rs17050543	rs2472032
rs2472039	rs1801128	rs17050545	rs4986999	rs2472040

rs3219014	rs1042294	rs2472033	rs2472041	rs9824261
rs2269112	rs2472034	rs4686370	rs1052133 **(Ser 326 Cys)**	rs17050547
rs3219003	rs2471899	rs11632	rs2472035	rs9818365
rs3895085	rs2619496	rs2075747	rs159153	rs415153
rs1091453	rs3218993	rs3219004	rs2471900	rs3218994
rs2471895	rs809256	rs3218995	rs2471896	rs812536
rs125701	rs2472036	rs2471901	rs3218996	rs2471897
rs786514	rs3218997	rs3219005	rs809784	rs3218999
rs159151	rs3219011	rs1801129	rs6787046	rs786513
rs1801126	rs2472037	rs786512	rs11548134	rs3219006
rs810862				

NAT2

rs11780272	rs1495744	rs2101857	rs7832071	rs13363820
rs1805158	rs6984200	rs1801279	rs13277605	rs1041983
rs9987109	rs1801280	rs7820330	rs4986996	rs7460995
rs12720065	rs2087852	rs4986997	rs2101684	rs1799929
rs7011792	rs1799930 **(Arg 197 Gln)**	rs1390358	rs923796	rs1208
rs4546703	rs1799931	rs4634684	rs2552	rs2410556
rs4646247	rs11996129	rs971473	rs4621844	rs721398
rs11785247	rs1115783	rs1115784	rs1961456	rs1112005
rs11782802	rs973874			

CYP2E1

rs7091961	rs12776213	rs1329148	rs10857736	rs12262150
rs6537611	rs10857732	rs10857737	rs9418989	rs6537612
rs10857733	rs12776473	rs10776686	rs10466129	rs11101801
rs10466130	rs4838767	rs11101810	rs9419081	rs9418990
rs9419082	rs10857738	rs11101803	rs11101811	rs10776687
rs3813865	rs4838688	rs3813866	rs11101805	rs11575869
rs2031918	rs8192766	rs2031919	rs11575870	rs11101806
rs6413423	rs4838689	rs3813867 **(1019 G/C Pst1)**	rs10857734	rs4838768
rs6413422	rs11101807	rs2031920 **(Rsa1 C/T)**	rs11101808	rs11101809
rs10857735				

IL18

rs187238	rs5744238	rs5744255	rs5744228	rs5744239
rs5744256	rs360718	rs7932965	rs5744257	rs360717
rs11214103	rs360720	rs5744229	rs5744241	rs5744258
rs100000353	rs5744242	rs5744259	rs5744231	rs5744243
rs5744260	rs5744232	rs5744244	rs5744261	rs7106524
rs360722	rs549908 **(105 A/C)**	rs189667	rs5023207	rs12290658
rs5744246	rs12271175	rs5744247	rs11606049	rs360721 **(−133 C/G)**
rs360716	rs360715	rs4988359	rs360714	rs12721559
rs2043055	rs5744248	rs5744233	rs5744249	rs795467
rs5744250	rs12270240	rs5744251	rs100000354	rs100000356
rs4937113	rs1834481	rs100000355	rs17215057	rs360723
rs5744253	rs5744237	rs5744254		

GSTM1

(Null genotype)	Not in LD

MMP1

rs529381	rs685265	rs1144396	rs7107224	rs504875
rs1155764	rs526215	rs534191	rs12280880	rs509332
rs542603	rs12283759	rs574939	rs2105581	rs573764
rs470206	rs7102189	rs533621	rs575727	rs1799750 **(-1607 G/GG)**
rs552306	rs634607	rs470211	rs12286876	rs470146

rs12285331	rs2075847	rs519806	rs473509	rs12283571
rs498186	rs2839969	rs2000609	rs7125865	rs570662
rs11225427	rs484915	rs470307	rs2408490	rs12279710

COX2

rs7527769	rs689465	rs4648270	rs7550380	rs12027712
rs12759220	rs2206594	rs689466	rs20430	rs6687495
rs2745558	rs4648271	rs6681231	rs3918304	rs11567825
rs13376484	rs20415	rs4648273	rs12064238	rs20416
rs16825748	rs10911911.	rs4648254	rs4648274	rs12743673
rs11567815	rs16825745	rs10911910	rs20417 **(-765G>C)**	rs20432
rs12743516	rs20433	rs10911909	rs4648256	rs3218622
rs1119066	rs20419	rs2066826	rs1119065	rs2734779
rs5278	rs1119064	rs20420	rs4648276	rs10798053
rs20422	rs20434	rs12409744	rs20423	rs3218623
rs10911908	rs5270	rs3218624	rs10911907	rs20424
rs5279	rs7416022	rs5271	rs4648278	rs2745561
rs4648257	rs13306034	rs10911906	rs11567819	rs2853803
rs2734776	rs3134591	rs4648279	rs2734777	rs3134592
rs4648281	rs12084433	rs20426	rs4648282	rs2734778
rs4648258	rs11567826	rs2745560	rs11567820	rs4648283
rs2223627	rs2745557	rs4648284	rs2383517	rs11567821
rs4648285	rs4295848	rs4648259	rs11567827	rs4428839
rs4648260	rs4648286	rs4609389	rs4648261	rs4648287
rs4428838	rs4648262	rs5272	rs12131210	rs11567822
rs4648288	rs2179555	rs11567823	rs5273	rs2143417
rs2066824	rs5274	rs2143416	rs20427	rs3218625
rs11583191	rs5277	rs4648289	rs2383516	rs2066823
rs4648290	rs2383515	rs4648263	rs1051896	rs10911905
rs4987012	rs5275	rs10911904	rs20428	rs6684912
rs20429	rs2745559	rs4648264	rs12042763	rs4648265
rs4648250	rs4648266	rs4648251	rs4648267	rs2223626
rs11567824	rs689462	rs4648268	rs4648253	rs4648269

IFNG

rs2069707	rs2069720	rs3814242	rs1042274	rs2069709
rs2069721	rs2069710	rs2069734	rs2069711	rs2069722
rs2069712	rs2234687	rs2430561 **(874 A/T)**	rs7957366	rs2069723
rs2069713	rs2069724	rs1861494	rs2069725	rs2234685
rs4394909	rs1861493	rs2069726	rs2069714	rs2069727
rs2069715	rs2069716	rs2069717	rs2069718	rs3087272
rs2069719	rs9282708			

CTGF

(-447 G/C), no rs number	Region of recombination

MUC5AC

(-221 C/T), no rs number	No LD data

MBL2

rs7899547	rs12264958	rs12255312	rs10824797	rs11003126
rs11003122	rs11003131	rs1031101	rs1982267	rs930506
rs10824795	rs1982266	rs930505	rs10824794	rs4935047
rs11003130	rs920725	rs4935046	rs2384044	rs7916582
rs10824793	rs2384045	rs920724	rs1838066	rs5027257
rs16933335	rs1838065	rs2384046	rs11003125	rs930509
rs12263867	rs7100749	rs930508	rs11003129	rs11003124
rs930507	rs12221393	rs7084554	rs2165811	rs7096206

rs12782244	rs11003123	rs11003128	rs11575988	rs17664818
rs11575989	rs7475766	rs7095891	rs10824796	rs4647963
rs16933417	rs8179079	rs2165810	rs5030737	rs11003127
rs1800450 **(161 G/A)**	rs3925313	rs7094151	rs1800451	rs7071882
rs12246310				

SOD3

rs1799895 **(Arg 231 Gly)**	Region of low LD

APEX1

rs3136820 **(Asp 148 Glu)**	Recombination region

NBS1

rs1805800	rs1805793	rs1805824	rs1805788	rs3026273
rs13312874	rs13312891	rs1805787	rs1805847	rs7463645
rs1805823	rs1805816	rs13312847	rs13312877	rs6990969
rs3026269	rs1805846	rs3026272	rs13312893	rs1805815
rs1805845	rs1805833	rs13312894	rs13312922	rs1805844
rs1805832	rs741777	rs1805786	rs13312849	rs7818138
rs13312895	rs709816	rs1805799	rs9792335	rs6985934
rs13312850	rs2339025	rs867185	rs13312851	rs3358
rs4596696	rs13312852	rs13277858	rs1805822	rs13312853
rs1805831	rs1805790	rs2073635	rs1805830	rs1569162
rs1063045	rs2293775	rs1805821	rs13312854	rs13259550
rs1805820	rs13312855	rs741778	rs1235369	rs1805798
rs1805829	rs3026270	rs1805797	rs1805828	rs1805789
rs2308961	rs769416	rs9694776	rs13312858	rs2272581
rs2293774	rs13312859	rs13312879	rs7008218	rs1805796
rs3026271	rs11998021	rs1805843	rs1805827	rs13312896
rs769417	rs13312880	rs13312897	rs769414	rs769418
rs12550313	rs1805842	rs769420	rs13312898	rs1805841
rs13312881	rs13312900	rs13312860	rs2308960	rs13312901
rs1805840	rs1805826	rs16786	rs13312861	rs10092465
rs13312902	rs1805839	rs13312883	rs1805819	rs1805838
rs1805792	rs2234744	rs13312862	rs13312884	rs13312903
rs13312863	rs13312885	rs1805818	rs1805837	rs13312886
rs7818042	rs13312864	rs13312887	rs13312904	rs13312865
rs1805791	rs7818989	rs13312866	rs1805825	rs13312905
rs13312867	rs13278453	rs13312906	rs11995115	rs13275276
rs13312907	rs1805836	rs10282890	rs9650096	rs1805795
rs16902052	rs9649958	rs7832009	rs7006322	rs7010210
rs1805835	rs7006318	rs13312913	rs13312871	rs6987873
rs2516635	rs13312872	rs9650098	rs13312916	rs1805794 **(Gln 185 Glu)**
rs13312888	rs11784904	rs13312889	rs13312921	rs1805834
rs13312890	rs1805817			

ARG1

rs2781659	rs2781660	rs2781661	rs2781662	rs2781663
rs2608898	rs2781664	rs3756780	rs2781665	rs2608897
rs2781666	rs17788484	rs9493029	rs2781667 **(Intron1 C/T)**	

REV1, REV1L

rs6714244	rs10172068	rs1000409	rs7597141	rs3208832
rs1839666	rs10180138	rs5013068	rs6714650	rs1801874
rs7560795	rs12465846	rs1451245	rs17022663	rs14534
rs10179697	rs9308821	rs3792141	rs17763586	rs2305353
rs4143760	rs10183488	rs3792142	rs13402784	rs2305352
rs10173883	rs7580448	rs3792143	rs7574943	rs12474305
rs10176205	rs7426356	rs3792144	rs4851205	rs13423815

rs12470050	rs13395100	rs2309585	rs7571793	rs17022639
rs7571186	rs11889026	rs7573502	rs3792152	rs7579403
rs13384316	rs13395095	rs10179435	rs10177775	rs12053237
rs6542882	rs11889025	rs7423755	rs3087394	rs12233126
rs13430962	rs2053917	rs1451244	rs3087398	rs11123786
rs13427780	rs13392042	rs3087386 **(Phe 257 Ser)**	rs2242037	rs11123785
rs6737560	rs13429806	rs11675410	rs3197957	rs1011633
rs10173466	rs3087391	rs17022653	rs2242036	rs6707685
rs10170425	rs3087399	rs12104881	rs17022634	rs13420087
rs10172752	rs10175852	rs12104509	rs3205290	rs6713792
rs10169151	rs12105223	rs12105686	rs7593274	rs7564587
rs4851206	rs7582085	rs12104601	rs13389623	rs11676988
rs7567674	rs6542880	rs13420293	rs11695517	rs7594838
rs11695422	rs3087383	rs2309604	rs13432046	rs7582133
rs3087385	rs6542881	rs10210346	rs13419448	rs3749087
rs896249	rs10210257	rs3087402	rs7560996	rs737094
rs13385501	rs3792146	rs7601730	rs11893335	rs2122748
rs3792147	rs13428419	rs13405400	rs13427424	rs3792148
rs3828316	rs13405256	rs10188633	rs13409359	rs717454
rs9308823	rs1451246	rs10496337	rs13394927	rs1973011
rs1839667	rs6542879	rs3087396	rs13398476	rs9973846
rs3792149	rs1053544	rs9308822	rs11899745	rs4341989
rs2290261	rs10191001	rs10865031	rs12479064	rs2290260
rs6746320	rs7572779	rs13429185	rs3087401	rs6728175
rs7563462	rs6717515	rs6711073	rs13415713	rs7563455
rs6727483	rs12465153	rs11902376	rs12986447	rs11887109
rs3087389	rs17022732	rs2053916	rs12619546	rs3087388
rs6748200	rs3792135	rs10182492	rs3209289	rs3792134
rs3792136	rs3792150	rs1046340	rs3087382	rs3792137
rs3792151	rs3087393	rs3087384	rs13416265	rs6723062
rs2305354	rs896248	rs3792138	rs1451243	rs3087392
rs959929	rs13387148	rs13426758	rs3087400	rs769105
rs1901283	rs9941566	rs3087387	rs7421436	rs10207435
rs7602535	rs3792153	rs13400661	rs7598629	rs3087395
rs2290259	rs7585019	rs3087403	rs17763718	rs2290258
rs13394688	rs3087390	rs4535093	rs9308819	rs10207981
rs10186223	rs10206944	rs2290257		

IGF2R

rs3798189	rs8191748	rs1570070	rs8191771	rs3798188
rs8191750	rs8191764	rs8191772	rs3798187	rs8191751
rs8191765	rs8191773	rs9365122	rs3798186	rs408889
rs13198308	rs9347380	rs6455680	rs8191766	rs8191774
rs6904531	rs8191752	rs8191767	rs8191775	rs2342868
rs11751626	rs8191768	rs2297358	rs12174439	rs8191753
rs2297357	rs3777413	rs12173776	rs8191754 **(Leu 252 Val)**	rs8191769
rs3798185	rs9347382	rs8191770	rs435612	rs9365124
rs2297356	rs448116	rs8191776	rs9457809	rs8191755
rs399919	rs6413489	rs12179343	rs8191756	rs1867348
rs894817	rs449276	rs8191757	rs7762627	rs9456496
rs9295121	rs7763887	rs9456497	rs9365125	rs11758686
rs9457811	rs8191758	rs681401	rs9457812	rs8191759
rs9456499	rs9295120	rs8191760	rs12662414	rs9456498
rs8191761	rs4709391	rs2277071	rs4709390	rs13218689
rs11759563	rs8191762	rs13220128	rs8191746	rs8191763
rs1036951	rs2277070	rs12374637	rs4709392	rs8191747
rs12374638	rs3777414			

IL10

rs1800896 **(-1082 A/G)**	rs3021098	rs5743626	rs3024509	rs2222202
rs1554286	rs5743627	rs1800894	rs3024490	rs2352792
rs3024495	rs1800871	rs3790622	rs3024492	rs5743628
rs1800895	rs3021093	rs3024507	rs3024496	rs1800872
rs3024491	rs3024493	rs3024497	rs3024488	rs3021094
rs1878672	rs3024510	rs3024489	rs1518110	rs3024508
rs3024498	rs5743625	rs3024506	rs9282740	rs3001099
rs1518111	rs3024494			

INDUSRIAL APPLICATION

The present invention is directed to methods for assessing a subject's risk of developing lung cancer. The methods comprise the analysis of polymorphisms herein shown to be associated with increased or decreased risk of developing lung cancer, 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 lung cancer 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.

Publications

Alberg AJ, Samet JM. Epidemiology of lung cancer. Chest 2003, 123, 21s-49s.

Anthonisen NR. Prognosis in COPD: results from multi-center clinical trials. Am Rev Respir Dis 1989, 140, s95-s99.

Cantlay AM, Smith CA, Wallace WA, Yap PL, Lamb D, Harrison DJ.Heterogeneous expression and polymorphic genotype of glutathione S-transferases in human lung. Thorax. 1994, 49(10):1010-4.

Kuller LH, et al. Relation of forced expiratory volume in one second to lung cancer mortality in the MRFIT. Am J Epidmiol 1190, 132, 265-274.

Mayne ST, et al. Previous lung disease and risk of lung cancer among men and women nonsmokers. Am J Epidemiol 1999, 149, 13-20.

Nomura a, et al. Prospective study of pulmonary function and lung cancer. Am Rev Respir Dis 1991, 144, 307-311.

Schwartz AG. Genetic predisposition to lung cancer. Chest 2004, 125, 86s-89s.

Skillrud DM, et al. Higher risk of lung cancer in COPD: a prospective matched controlled study. Ann Int Med 1986, 105, 503-507.

Tockman MS, et al. Airways obstruction and the risk for lung cancer. Ann Int Med 1987, 106, 512-518.

Wu X, Zhao H, Suk R, Christiani DC. Genetic susceptibility to tobacco-related cancer. Oncogene 2004, 23, 6500-6523.

***

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 lung cancer comprising analysing a sample from said subject for the presence or absence of one or more polymorphisms selected from the group consisting of:
   Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);
   -786 T/C in the promoter of the gene encoding Nitric oxide synthase 3;
   Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);
   Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);
   Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);
   105 A/C in the gene encoding Interleukin-18 (IL-18);
   -133 G/C in the promoter of the gene encoding Interleukin-18;
   874 A/T in the gene encoding Interferon gamma (IFNγ);
   -765 G/C in the gene encoding Cyclooxygenase 2 (COX2);
   -447 G/C in the gene encoding Connective tissue growth factor (CTGF);
   -221 C/T in the gene encoding Mucin 5AC (MUC5AC);
   +161 G/A in the gene encoding Mannose binding lectin 2 (MBL2);
   intron 1 C/T in the gene encoding Arginase 1 (Arg1);
   Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);
   -1082 A/G in the gene encoding Interleukin 10 (IL-10);
   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 lung cancer.
2. A method according to claim 1 wherein the presence of one or more of the polymorphisms selected from the group consisting of:
   the Asp 298 Glu TT genotype in the gene encoding NOS3;
   the Arg 312 Gln CG or GG genotype in the gene encoding SOD3;
   the Asn 357 Ser AG or GG genotype in the gene encoding MMP12;
   the 105 AC or CC genotype in the gene encoding IL-18;
   the -133 CG or GG genotype in the gene encoding IL-18;
   the -765 CC or CG genotype in the promoter of the gene encoding COX2;
   the -221 TT genotype in the gene encoding MUC5AC;

the intron 1 C/T TT genotype in the gene encoding Arg1;

the Leu252Val GG genotype in the gene encoding IGF2R; or

the –1082 GG genotype in the gene encoding IL-10;

is indicative of a reduced risk of developing lung cancer.

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

the -786 TT genotype in the promoter of the gene encoding NOS3;

the Ala 15 Thr GG genotype in the gene encoding ACT;

the 105 AA genotype in the gene encoding IL-18;

the -133 CC genotype in the promoter of the gene encoding IL-18;

the 874 AA genotype in the gene encoding IFNγ;

the -765 GG genotype in the promoter of the gene encoding COX2;

the -447 CC or GC genotype in the gene encoding CTGF; or

the +161 AA or AG genotype in the gene encoding MBL2;

is indicative of an increased risk of developing lung cancer.

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:

Arg 399 Gln G/A in the X-ray repair complementing defective in Chinese hamster 1 (XRCC1) gene;

-251 A/T in the gene encoding Interleukin-8 (IL-8);

A870G in the gene encoding Cyclin D (CCND1);

-511 A/G in the gene encoding Interleukin 1B (IL-1B);

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the Xeroderma pigmentosum complementation group D (XPD) gene;

Ile 462 Val A/G in the gene encoding Cytochrome P450 1A1 (CYP1A1);

Ser 326 Cys G/C in the gene encoding 8-Oxoguanine DNA glycolase (OGG1);

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

1019 G/C Pst I in the gene encoding Cytochrome P450 2E1 (CYP2E1);

C/T Rsa I in the gene encoding Cytochrome P450 2E1;

GSTM null in the gene encoding Glutathione S-transferase M (GSTM);

-1607 1G/2G in the promoter of the gene encoding Matrix metalloproteinase 1 (MMP1);

Gln 185 Glu G/C in the gene encoding Nibrin (NBS1);

Phe 257 Ser C/T in the gene encoding REV1;

Asp 148 Glu G/T in the gene encoding Apex nuclease (APE1); 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 Asp 298 Glu TT genotype in the gene encoding NOS3;

the Arg 312 Gln CG or GG genotype in the gene encoding SOD3;

the Asn 357 Ser AG or GG genotype in the gene encoding MMP12;

the 105 AC or CC genotype in the gene encoding IL-18;

the -133 CG or GG genotype in the gene encoding IL-18;

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

the -221 TT genotype in the gene encoding MUC5AC;

the intron 1 C/T TT genotype in the gene encoding Arg1;

the Leu252Val GG genotype in the gene encoding IGF2R;

the –1082 GG genotype in the gene encoding IL-10

the -251 AA genotype in the gene encoding IL-8;

the Arg 399 Gln AA genotype in the XRCC1 gene ;

the A870G GG genotype in the gene encoding CCND1;

the -751 GG genotype in the promoter of the XPD gene ;

the Ile 462 Val AG or GG genotype in the gene encoding CYP1A1;

the Ser 326 Cys GG genotype in the gene encoding OGG1; or

the Phe 257 Ser CC genotype in the gene encoding REV1;

is indicative of a reduced risk of developing lung cancer.

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 -786 TT genotype in the promoter of the gene encoding NOS3;

the Ala 15 Thr GG genotype in the gene encoding ACT;

the 105 AA genotype in the gene encoding IL-18;

the -133 CC genotype in the promoter of the gene encoding IL-18;

the 874 AA genotype in the gene encoding IFNγ;

the -765 GG genotype in the promoter of the gene encoding COX2;

the -447 CC or GC genotype in the gene encoding CTGF;

the +161 AA or AG genotype in the gene encoding MBL2;

the -511 GG genotype in the gene encoding IL-1B;

the A-670G AA genotype in the gene encoding FAS;

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

the Ile462 Val AA genotype in the gene encoding CYP1A1;

the 1019 G/C Pst I CC or CG genotype in the gene encoding CYP2E1;

the C/T Rsa I TT or TC genotype in the gene encoding CYP2E1;

the GSTM null genotype in the gene encoding GSTM;

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

the Gln 185 Glu CC genotype in the gene encoding NBS1; or

the Asp 148 Glu GG genotype in the gene encoding APE1;

is indicative of an increased risk of developing lung cancer.

7. A method of assessing a subject's risk of developing lung cancer said method comprising the steps:

(i) determining the presence or absence of at least one protective polymorphism associated with a reduced risk of developing lung cancer; 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 lung cancer;

wherein the presence of one or more of said protective polymorphisms is indicative of a reduced risk of developing lung cancer, 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 lung cancer.

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

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

the Arg 312 Gln CG or GG genotype in the gene encoding SOD3;

the Asn 357 Ser AG or GG genotype in the gene encoding MMP12;

the 105 AC or CC genotype in the gene encoding IL-18;

the -133 CG or GG genotype in the gene encoding IL-18;

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

the -221 TT genotype in the gene encoding MUC5AC;

the intron 1 C/T TT genotype in the gene encoding Arg1;

the Leu252Val GG genotype in the gene encoding IGF2R;

the –1082 GG genotype in the gene encoding IL-10;

the -251 AA genotype in the gene encoding IL-8;

the Arg 399 Gln AA genotype in the XRCC1 gene;

the A870G GG genotype in the gene encoding CCND1;

the -751 GG genotype in the promoter of the XPD gene ;

the Ile 462 Val AG or GG genotype in the gene encoding CYP1A1;

the Ser 326 Cys GG genotype in the gene encoding OGG1; or

the Phe 257 Ser CC genotype in the gene encoding REV1.

9. A method according to claim 7 or 8 wherein said at least one susceptibility polymorphism is a genotype selected from the group consisting of:

the -786 TT genotype in the promoter of the gene encoding NOS3;

the Ala 15 Thr GG genotype in the gene encoding ACT;

the 105 AA genotype in the gene encoding IL-18;

the -133 CC genotype in the promoter of the gene encoding IL-18;

the 874 AA genotype in the gene encoding IFNγ;

the -765 GG genotype in the promoter of the gene encoding COX2;

the -447 CC or GC genotype in the gene encoding CTGF;

the +161 AA or AG genotype in the gene encoding MBL2;

the -511 GG genotype in the gene encoding IL-1B;

the A-670G AA genotype in the gene encoding FAS;

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

the Ile462 Val AA genotype in the gene encoding CYP1A1;

the 1019 G/C Pst I CC or CG genotype in the gene encoding CYP2E1;

the C/T Rsa I TT or TC genotype in the gene encoding CYP2E1;

the GSTM null genotype in the gene encoding GSTM;

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

the Gln 185 Glu CC genotype in the gene encoding NBS1; or

the Asp 148 Glu GG genotype in the gene encoding APE1

10. A method according to any one of claims 7 to 9 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 lung cancer.

11. A method according to any one of claims 7 to 9 wherein in the absence of a protective polymorphism the presence of one or more susceptibility polymorphisms is indicative of an increased risk of developing lung cancer.

12. A method according to any one of claims 7 to 9 wherein the presence of two or more susceptibility polymorphisms is indicative of an increased risk of developing lung cancer.

13. A method of determining a subject's risk of developing lung cancer, comprising analysing a sample from said subject for the presence of two or more polymorphisms selected from the group consisting of:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);

-1082 A/G in the gene encoding Interleukin 10 (IL-10);

Arg 399 Gln G/A in the X-ray repair complementing defective in Chinese hamster 1 (XRCC1) gene;

-251 A/T in the gene encoding Interleukin-8 (IL-8);

A870G in the gene encoding Cyclin D (CCND1);

-511 A/G in the gene encoding Interleukin 1B (IL-1B);

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the Xeroderma pigmentosum complementation group D (XPD) gene;

Ile 462 Val A/G in the gene encoding Cytochrome P450 1A1 (CYP1A1);

Ser 326 Cys G/C in the gene encoding 8-Oxoguanine DNA glycolase (OGG1);

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

1019 G/C Pst I in the gene encoding Cytochrome P450 2E1 (CYP2E1);

C/T Rsa I in the gene encoding Cytochrome P450 2E1;

GSTM null in the gene encoding Glutathione S-transferase M (GSTM);

-1607 1G/2G in the promoter of the gene encoding Matrix metalloproteinase 1 (MMP1);

Gln 185 Glu G/C in the gene encoding Nibrin (NBS1);

Phe 257 Ser C/T in the gene encoding REV1;

Asp 148 Glu G/T in the gene encoding Apex nuclease (APE1);

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

14. A method according to any one of claims 1 to 13 wherein said method comprises the analysis of one or more epidemiological risk factors.

15. A method of determining a subject's risk of developing lung cancer, 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:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);

-1082 A/G in the gene encoding Interleukin 10 (IL-10);

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 lung cancer.

16. A method according to claim 15 wherein a result indicating the presence of one or more of the polymorphisms selected from the group consisting of:

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

the Arg 312 Gln CG or GG genotype in the gene encoding SOD3;

the Asn 357 Ser AG or GG genotype in the gene encoding MMP12;

the 105 AC or CC genotype in the gene encoding IL-18;

the -133 CG or GG genotype in the gene encoding IL-18;

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

the -221 TT genotype in the gene encoding MUC5AC;

the intron 1 C/T TT genotype in the gene encoding Arg1;

the Leu252Val GG genotype in the gene encoding IGF2R; or

the –1082 GG genotype in the gene encoding IL-10;

is indicative of a reduced risk of developing lung cancer.

17. A method according to claim 15 wherein a result indicating the presence of one or more of the polymorphisms selected from the group consisting of;

the -786 TT genotype in the promoter of the gene encoding NOS3;

the Ala 15 Thr GG genotype in the gene encoding ACT;

the 105 AA genotype in the gene encoding IL-18;

the -133 CC genotype in the promoter of the gene encoding IL-18;

the 874 AA genotype in the gene encoding IFNγ;

the -765 GG genotype in the promoter of the gene encoding COX2;

the -447 CC or GC genotype in the gene encoding CTGF; or

the +161 AA or AG genotype in the gene encoding MBL2;

is indicative of an increased risk of developing lung cancer.

18. One or more nucleotide probes and/or primers for use in the method of any one of claims 1 to 17 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. One or more nucleotide probes and/or primers as claimed in claim 18 comprising the sequence of any one of SEQ.ID.NO.1 to SEQ.ID.NO.145.

20. 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.

21. The use of at least one polymorphism in the assessment of a subject's risk of developing lung cancer, wherein said at least one polymorphism is selected from the group consisting of:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);or

-1082 A/G in the gene encoding Interleukin 10 (IL-10);

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

22. The use according to claim 21, wherein said use is in conjunction with the use of at least one further polymorphism selected from the group consisting of:

Arg 399 Gln G/A in the X-ray repair complementing defective in Chinese hamster 1 (XRCC1) gene;

-251 A/T in the gene encoding Interleukin-8 (IL-8);

A870G in the gene encoding Cyclin D (CCND1);

-511 A/G in the gene encoding Interleukin 1B (IL-1B);

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the Xeroderma pigmentosum complementation group D (XPD) gene;

Ile 462 Val A/G in the gene encoding Cytochrome P450 1A1 (CYP1A1);

Ser 326 Cys G/C in the gene encoding 8-Oxoguanine DNA glycolase (OGG1);

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

1019 G/C Pst I in the gene encoding Cytochrome P450 2E1 (CYP2E1);

C/T Rsa I in the gene encoding Cytochrome P450 2E1;

GSTM null in the gene encoding Glutathione S-transferase M (GSTM);

-1607 1G/2G in the promoter of the gene encoding Matrix metalloproteinase 1 (MMP1);

Gln 185 Glu G/C in the gene encoding Nibrin (NBS1);

Phe 257 Ser C/T in the gene encoding REV1;

Asp 148 Glu G/T in the gene encoding Apex nuclease (APE1);

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

23. A method of treating a subject having an increased risk of developing lung cancer 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.

24. A method of treating a subject having an increased risk of developinglung cancer, said subject having a detectable susceptibility polymorphism selected from the group defined in claim 9 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.

25. A method of treating a subject having an increased risk of developing lung cancer 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.

26. A method of treating a subject having an increased risk of developing lung cancer 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.

27. A method of determining a subject's risk of developing lung cancer , comprising the analysis of two or more polymorphisms selected from the group consisting of:

Asp 298 Glu in the gene encoding NOS3;

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding SOD3;

-251 A/T in the gene encoding IL-8;

Ala 15 Thr in the gene encoding ACT;

Asn 357 Ser A/G in the gene encoding MMP12;

105 A/C in the gene encoding IL-18;

-133 G/C in the promoter of the gene encoding IL-18;

874 A/T in the gene encoding IFNγ;

Arg 399 Gln G/A in the XRCC1 gene;

A870G in the gene encoding CCND1;

-511 A/G in the gene encoding IL-1B;

-670G in the gene encoding FAS (Apo-1/CD95);

-751 G/T in the promoter of the XPD gene;

Ile 462 Val A/G in the gene encoding CYP1A1;

Ser 326 Cys G/C in the gene encoding OGG1;

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

1019 G/C Pst I in the gene encoding CYP2E1;

C/T Rsa I in the gene encoding CYP2E1;

GSTM null in the gene encoding GSTM;

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

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

-447 G/C in the gene encoding CTGF;

-221 C/T in the gene encoding MUC5AC;

+161 G/A in the gene encoding MBL2;

intron 1 C/T in the gene encoding Arg1;

Leu 252 Val C/G in the gene encoding IGF2R;

-1082 A/G in the gene encoding IL-10;

Gln 185 Glu G/C in the gene encoding NBS1;

Phe 257 Ser C/T in the gene encoding REV1;

Asp 148 Glu G/T in the gene encoding APE1; or

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

28. An antibody microarray for use in the methods as claimed in any one of claims 1 to 17 or 27, 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 defined in claim 2 or 3.

29. 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 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.

30. A method according to claim 29 wherein said cell is a human lung cell which has been pre-screened to confirm the presence of said polymorphism.

31. A method according to claim 29 or 30 wherein 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.

32. A method according to claim 29 or 30 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.

33. A method according to claim 29 or 30 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.

34. A method according to claim 29 or 30 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.

35. 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 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.

36. A method according to claim 35 wherein said cell is a human lung cell which has been pre-screened to confirm the presence, and baseline level of expression, of said gene.

37. A method according to claim 35 or 36 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.

38. A method according to claim 35 or 36 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.

39. A method according to claim 35 or 36 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.

40. A method according to claim 35 or 36 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.

41. A method of assessing the likely responsiveness of a subject predisposed to or diagnosed with lung cancer 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.

42. A method of treating a subject having an increased risk of developing lung cancer 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.

43. A method according to claim 42 wherein said agent is a COX2 inhibitor or a nonsteroidal anti-inflammatory drug (NSAID).

44. A method according to claim 43 wherein said COX2 inhibitor is selected from the group consisting of Celebrex (Celecoxib), Bextra (Valdecoxib), and Vioxx (Rofecoxib).

45. A method of treating a subject having an increased risk of developing lung cancer and for whom the presence of the 2G2G genotype at the -1607 1G/2G polymorphism in the promoter of the gene encoding MMP1 has been determined, said method comprising administering to said subject an agent capable of reducing MMP1 activity in said subject.

46. A method according to claim 45 wherein said agent is a MMP inhibitor.

47. A method according to claim 46 wherein said MMP inhibitor is selected from the group comprising 4,5-dihydroxyanthaquinone-2-carboxylic acid (AQCA), anthraquinyl-mercaptoethyamine, anthraquinyl-alanine hydroxamate, and derivatives thereof.

48. A method according to claim 45 wherein said agent is an agent capable of increasing expression of or the activity of one or more tissue inhibitors of metalloproteinases (TIMPs).

49. A method according to claim 48 wherein said tissue inhibitors of metalloproteinases is one or more of TIMP1, TIMP2, TIMP3, or TIMP4.

50. A method according to claim 45 wherein said agent is an agent capable of reducing expression of or the activity of one or more membrane bound MMPs.

51. A kit for assessing a subject's risk of developing one or more obstructive lung diseases selected from lung cancer, 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:

Asp 298 Glu in the gene encoding Nitric oxide synthase 3 (NOS3);

-786 T/C in the promoter of the gene encoding NOS3;

Arg 312 Gln in the gene encoding Superoxide dismutase 3 (SOD3);

Ala 15 Thr in the gene encoding Anti-chymotrypsin (ACT);

Asn 357 Ser A/G in the gene encoding Matrix metalloproteinase 12 (MMP12);

105 A/C in the gene encoding Interleukin-18 (IL-18);

-133 G/C in the promoter of the gene encoding Interleukin-18;

874 A/T in the gene encoding Interferon gamma (IFNγ);

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

-447 G/C in the gene encoding Connective tissue growth factor (CTGF);

-221 C/T in the gene encoding Mucin 5AC (MUC5AC);

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

intron 1 C/T in the gene encoding Arginase 1 (Arg1);

Leu 252 Val C/G in the gene encoding Insulin-like growth factor II receptor (IGF2R);

-1082 A/G in the gene encoding Interleukin 10 (IL-10);

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