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(57) Abstract: The present invention provides systems, methods, and classifiers for differentiating between samples as usual interstitial pneumonia (UIP) or non-UIP.

WO 2016/073768 A1

SYSTEMS AND METHODS OF DIAGNOSING IDIOPATHIC PULMONARY FIBROSIS ON TRANSBRONCHIAL BIOPSIES USING MACHINE LEARNING AND HIGH DIMENSIONAL TRANSCRIPTIONAL DATA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional application Serial No. 62/075,328, filed November 5, 2014, and U.S. Provisional application Serial No. 62/130,800, filed March 10, 2015, each of which is incorporated by reference herein in its entirety. This application also incorporates by reference herein in its entirety the entire subject matter of PCT/US2014/029029, filed March 14, 2014.

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INTRODUCTION

[0003] Interstitial lung diseases (ILD) are a heterogeneous group of acute and chronic bilateral parenchymal pulmonary disorders with similar clinical manifestations, but a wide spectrum of severity and outcome [1,2]. Among these, idiopathic pulmonary fibrosis (IPF) is one of the most common and severe ILD, characterized by progressive fibrosis, worsening lung function and death [3-6]. Most patients diagnosed with IPF die within five years of their initial diagnosis[7,8]. However the recent availability of two new drugs and other therapeutics in development may change this picture[9-11], and accurate diagnosis is critical for appropriate therapeutic intervention [5,12].

[0004] IPF can be challenging to diagnose. The diagnostic approach to IPF requires exclusion of other interstitial pneumonias, as well as connective tissue disease and environmental and occupational exposures[3-6]. Patients suspected of having IPF usually undergo high-resolution computed tomography (HRCT), which confirms the disease with high specificity only if the pattern of usual interstitial pneumonia (UIP) is clearly evident [5,13]. Yet, for a large number of patients, diagnosis necessitates an invasive surgical lung biopsy (SLB) to clarify the histopathologic features of interstitial pneumonia and/or UIP pattern[5,14] and the typical length of time to diagnose IPF from the onset of symptoms may be 1-2 years

[15]. Discordance between pathologists occurs, and a correct diagnosis can be dependent on individual experience[16]. Despite histopathologic evaluation, a definitive diagnosis may remain elusive. Diagnostic accuracy has been shown to increase when multidisciplinary teams (MDT) of pulmonologists, radiologists, and pathologists confer [17]; unfortunately not all patients and their physicians have access to this level of expert review by an experienced MDT. Such reviews are time consuming and require patients to be seen at regional centers of recognized expertise.

[0005] Accordingly, more effective methods of diagnosing IPF are required. In addition, methods of differentiating UIP from non-UIP are required.

SUMMARY OF THE INVENTION

[0006] Herein we describe methods of and systems used for differentiating between samples as usual interstitial pneumonia (UIP) or non-UIP using classifiers whose accuracy was confirmed using expert pathology diagnoses as truth labels. While gene expression profiling studies in the scientific literature have reported differential expression between IPF and other ILD subtypes[18,19], none have attempted to classify UIP in datasets containing other subtypes frequently present as part of the clinician's differential diagnosis.

[0007] In some embodiments, the present invention provides a method and/or system for detecting whether a lung tissue sample is positive for usual interstitial pneumonia (UIP) or non-usual interstitial pneumonia (non-UIP). In some embodiments a method is provided for: assaying the expression level of each of a first group of transcripts and a second group of transcripts in a test sample of a subject, wherein the first group of transcripts includes any one or more of the genes overexpressed in UIP and listed in any of Tables 5, 7, 9, 10, 11, and 12 and the second group of transcripts includes any one or more of the genes under-expressed in UIP and listed in any of Tables 5, 8, 9, 10, 11 or 12. In some embodiment, the method further provides forcomparing the expression level of each of the first group of transcripts and the second group of transcripts with reference expression levels of the corresponding transcripts to (1) classify said lung tissue as usual interstitial pneumonia (UIP) if there is (a) an increase in an expression level corresponding to the first group or (b) a decrease in an expression level corresponding to the second group as compared to the reference expression levels, or (2) classify the lung tissue as non-usual interstitial pneumonia (non-UIP) if there is (c) an increase in the expression level corresponding to the second group or (d) a decrease in the expression level corresponding to the first group as compared to the reference expression

levels. In some embodiments, the method further provides for determining and/or comparing sequence variants for any of the one or more genes listed in tables 5, 8, 9, 11, and/or 12.

[0008] In some embodiments, the present invention provides a method and/or system for detecting whether a lung tissue sample is positive for usual interstitial pneumonia (UIP) or non-usual interstitial pneumonia (non-UIP). In some embodiments, the method and/or system is used to assay by sequencing, array hybridization, or nucleic acid amplification the expression level of each of a first group of transcripts and a second group of transcripts in a test sample from a lung tissue of a subject, wherein the first group of transcripts includes any one or more of the genes over-expressed in UIP and listed in Tables 5, 7, 9, 10, 11 or 12 and the second group of transcripts includes any one or more of the genes under-expressed in UIP and listed in Tables 5, 8, 9, 10, 11 or 12. In certain embodiments, the method and/or system furthercompares the expression level of each of the first group of transcripts and the second group of transcripts with reference expression levels of the corresponding transcripts to (1) classify said lung tissue as usual interstitial pneumonia (UIP) if there is (a) an increase in an expression level corresponding to the first group or (b) a decrease in an expression level corresponding to the second group as compared to the reference expression levels, or (2) classify the lung tissue as non-usual interstitial pneumonia (non-UIP) if there is (c) an increase in the expression level corresponding to the second group or (d) a decrease in the expression level corresponding to the first group as compared to the reference expression levels.

[0009] In some embodiments, the present invention provides a method and/or system for detecting whether a test sample is positive for UIP or non-UIP by

measuring the expression level of two or more transcripts expressed and/or determining sequence variants for one or more transcripts expressed in the sample;

using a computer generated classifier to distinguish between UIP and non-UIP;

wherein the classifier is built using a spectrum of Non-UIP pathology subtypes comprising HP, NSIP, sarcoidosis, RB, bronchiolitis, and organizing pneumonia (OP).

[0010] In some embodiments, the test sample is a biopsy sample or a bronchoalveolar lavage sample. In some embodiments, the test sample is fresh-frozen or fixed.

[0011] In some embodiments, the transcript expression levels are determined by RT-PCR, DNA microarray hybridization, RNASeq, or a combination thereof. In some embodiments, one or more of the transcripts is labeled.

[0012] In some embodiments, the method comprises detecting cDNA produced from RNA expressed in the test sample, wherein, optionally, the cDNA is amplified from a plurality of cDNA transcripts prior to the detecting step.

[0013] In some embodiments, the methods of the present invention further comprise measuring the expression level of at least one control nucleic acid in the test sample.

[0014] In some embodiments, the methods of the present invention classify the lung tissue as any one of interstitial lung diseases (ILD), a particular type of ILD, a non-ILD, or non-diagnostic. In particular embodiments, methods of the present invention classify the lung tissue as either idiopathic pulmonary fibrosis (IPF) or Nonspecific interstitial pneumonia (NSIP).

[0015] In some embodiments, the method and/or system of the present invention comprises assaying the test sample for the expression level of one or more transcripts of any one of SEQ ID NOS: 1-22. In some embodiments, the method further comprises assaying the test sample for the expression level of from 1 to 20 other genes. In some embodiments, the other genes comprise one or more, or optionally all of HMCN2, ADAMTSL1, CD79B, KEL, KLHL14, MPP2, NMNAT2, PLXDC1, CAPN9, TALDO1, PLK4, IGHV3-72, IGKV1-9, and CNTN4.

[0016] In some embodiments, the method and/or systems of the present invention further comprise using smoking status as a covariate during training of a UIP vs. non-UIP classifier disclosed herein, wherein, optionally, the smoking status is determined by detecting an expression profile indicative of the subject's smoker status. In some embodiments, such a classifier is used to determine whether a test sample is UIP or non-UIP.

[0017] In some embodiments, the method and/or systems of the present invention comprises training a UIP vs. non-UIP classifier, wherein genes that are susceptible to smoker-status bias are excluded or weighed differently than genes that are not susceptible to smoker-status bias during the classifier training.

[0018] In some embodiments, the present invention provides a method and/or system for detecting whether a lung tissue sample is positive for usual interstitial pneumonia (UIP) or

non-usual interstitial pneumonia (non-UIP), as described herein, wherein the method comprises a first classification of a test sample as smoker or non-smoker using a first classifier trained to recognize gene signatures that distinguish smokers from non-smokers; and wherein the method further comprises a second classification of the test sample a UIP or non-UIP, wherein the second classification step uses a second or third classifier, which second and third classifiers are trained to distinguish UIP vs. non-UIP in smokers (smoker-specific classifier) and non-smokers (non-smoker-specific classifier), respectively, and wherein the second classification uses either (i) the smoker-specific classifier if the test sample is classified as smoker in the first classification or (ii) the non-smoker-specific classifier if the test sample is classified as non-smoker in the first classification.

[0019] In some embodiments, the present invention provides a method and/or system for detecting whether a lung tissue sample is positive for usual interstitial pneumonia (UIP) or non-usual interstitial pneumonia (non-UIP), wherein the methods comprise implementing a classifier trained using one or more feature selected from gene expression, variants, mutations, fusions, loss of heterozygoxity (LOH), and biological pathway effect. In some embodiments, the classifier is trained using features comprising gene expression, sequence variants, mutations, fusions, loss of heterozygoxity (LOH), and biological pathway effect.

[0020] In some embodiments, the present invention provides for assaying 2 or more different transcripts, or 3 or more, 4 or more, 5 or more, 10 or more, 15 or more, 20 or more, or more than 20 different transcripts in the first group and/or 2 or more different transcripts, or 3 or more, 4 or more, 5 or more, 10 or more, 15 or more, 20 or more, or more than 20 different transcripts in the second group.

[0021] In some embodiments, the method provides for detecting 2 or more different transcripts of any one of SEQ ID NOS:1-22, or 3 or more, 4 or more, 5 or more, 10 or more, 15 or more, 20 or more, or more than 20 different transcripts of any one of SEQ ID NOS:1-22. In particular embodiments, the current methods provide for assaying the test sample for the expression level of all of the transcripts of SEQ ID NOS: 1-22. In some embodiments, the method further comprising assaying the test sample for the expression level of from 1 to 20 other genes. In some embodiments, the method provides for assaying one or more of HMCN2, ADAMTSL1, CD79B, KEL, KLHL14, MPP2, NMNAT2, PLXDC1, CAPN9, TALDO1, PLK4, IGHV3-72, IGKV1-9, and CNTN4.

BRIEF DESCRIPTION OF THE FIGURES

[0022] Fig. 1. Pairwise correlation on explant samples obtained from three patients diagnosed with IPF (Patients P1, P2, and P3). Locations (upper or lower, central or peripheral) are indicated for each sample. The top 200 differentially expressed genes separating IPF samples from normal lung samples were used to compute pairwise Pearson correlation coefficients and plotted as a heatmap with higher correlation represented in magenta color, and lower correlation represented in green color. Correlation between and with normal lung samples are in the 0·7 range (not shown).

[0023] Figs. 2A-2D. Performance of a classifier built using microarray data. ROC curves were used to characterize performance in the training set using leave-one-patient-out (LOPO) cross-validation (Fig. 2A) and in the independent test set by scoring the samples with a fixed model (Fig. 2C). Scores for individual samples are shown across patients in the training set (Fig. 2B), and across patients in an independent test set (Fig. 2D). Patient-level pathology diagnosis is shown on the x-axis. Samples with UIP pathology labels are indicated by closed circles, and non-UIP samples by pathology are shown in open triangles. A dotted horizontal line is drawn to indicate the threshold that corresponds to 92% specificity and 64% sensitivity (Fig. 2B) and 92% specificity and 82% sensitivity (Fig. 2D).

[0024] Figs. 3A-3D. Performance of classifiers built using RNASeq (Fig. 3A and Fig.3B) and microarray on the matched set (Fig. 3C and Fig.3D). Leave-one-patient-out (LOPO) cross-validation was performed and receiver operator characteristic (ROC) curves were produced for RNASeq (Fig. 3A) and microarray (Fig. 3C) classifiers. Scores for individual samples in the training sets are shown for RNASeq (Fig. 3B), and microarray (Fig. 3D) classification. Patient-level pathology diagnosis is shown on the x-axis. Samples with UIP pathology labels are indicated by closed circles, and non-UIP samples by pathology are shown in open triangles. A score threshold corresponding to 95% specificity is indicated as a horizontal line in Fig. 3B and Fig. 3D.

[0025] Fig. 4. Simulation study assessing the impact of mislabeling on the classification performance. The array training set (n=77) was used for this study. At a given proportion of swapped labels in the data set (x-axis), individual samples' classification labels were swapped to another class label with a weight accounting for the disagreement level of three expert pathology diagnoses. Each boxplot was drawn using LOPO CV performances (AUC) from 100 repeated simulations. The finer dotted horizontal line at AUC=0.5 represents random

performance, i.e., no classification, and the coarser dotted line corresponds to the classifier performance shown in Fig. 2A.

[0026] Fig. 5. Central pathology diagnostic process for a hypothetical patient with two samples (sample A and sample B). Three expert pathologists participate in the review process. For sample-level diagnosis, the glass slides for each sample are reviewed by each pathologist (Pathologist is abbreviated as Path.). For patient-level diagnosis, glass slides from all samples (two in this exercise) are gathered and reviewed together by each pathologist. Both sample-level and patient-level diagnoses go through the same review process. A majority vote is used as the final diagnosis, unless expert pathologists disagree even after the conferral, in which case, the sample is omitted due to lack of confidence in the diagnosis. Only a single such case was observed among all banked tissues (n=128).

[0027] Fig. 6. Location of lung samplings from three normal organ donors (top) and three patients diagnosed with IPF (bottom). Donors N1-N3 and P3 were female. Donors P1 and P2 were male.

[0028] Fig. 7A. Illustration of a computer system usable for implementing aspects disclosed herein.

[0029] Fig. 7B. Detailed illustration of the processor of the computer system of Fig. 7A.

[0100] Fig. 7C. Detailed illustration of one non-limiting method of the present invention, wherein gene product expression data for known UIP and non-UIP samples are used to train a classifier (e.g., using a classifier training module) for differentiating UIP vs. non-UIP, wherein the classifier optionally considers smoker status as a covariant, and wherein gene product expression data from unknown samples are input into the trained classifier to identify the unknown samples as either UIP or non-UIP, and wherein the results of the classification via the classifier are defined and output via a report.

[0030] Fig. 8. Differential gene expression in UIP and Non-UIP samples between smokers and non-smokers. The number of genes differentially expressed between UIP and Non UIP samples differs drastically between smokers and non-smokers.

[0031] Fig. 9. Shows differential gene expression between UIP and Non-UIP samples is susceptible to smoker-status bias. Direction (*i.e.*, over- vs. under-expression) and magnitude (circle size) of differential gene expression is confounded by smoking status.

[0032] Figs. 10A-10D. Examples of genes that are differentially expressed in UIP vs. Non-UIP and the effect of smoking status on expression levels. Fig. 10A: differential expression of IGHV3-72 in UIP vs Non-UIP smokers vs. non-smokers. Fig. 10B: differential expression of CPXM1 in UIP vs Non-UIP smokers vs. non-smokers. Fig. 10C: differential expression of BPIFA1 in UIP vs Non-UIP smokers vs. non-smokers. Fig. 10D: differential expression of HLA-U in UIP vs Non-UIP smokers vs. non-smokers.

DEFINITIONS

[0033] "Interstitial lung disease" or "ILD" (also known as diffuse parenchymal lung disease (DPLD)) as used herein refers to a group of lung diseases affecting the interstitium (the tissue and space around the air sacs of the lungs). ILD can be classified according to a suspected or known cause, or can be idiopathic. For example, ILD can be classified as caused by inhaled substances (inorganic or organic), drug induced (e.g., antibiotics, chemotherapeutic drugs, antiarrhythmic agents, statins), associated with connective tissue disease (e.g., systemic sclerosis, polymyositis, dermatomyositis, systemic lupus erythematous, rheumatoid arthritis), associated with pulmonary infection (e.g., atypical pneumonia, Pneumocystis pneumonia (PCP), tuberculosis, Chlamydia trachomatis, Respiratory Syncytial Virus), associated with a malignancy (e.g., Lymphangitic carcinomatosis), or can be idiopathic (e.g., sarcoidosis, idiopathic pulmonary fibrosis, Hamman-Rich syndrome, antisynthetase syndrome).

[0034] "ILD Inflammation" as used herein refers to an analytical grouping of inflammatory ILD subtypes characterized by underlying inflammation. These subtypes can be used collectively as a comparator against IPF and/or any other non-inflammation lung disease subtype. "ILD inflammation" can include HP, NSIP, sarcoidosis, and/or organizing pneumonia.

[0035] "Idiopathic interstitial pneumonia" or "IIP" (also referred to as noninfectious pneumonia" refers to a class of ILDs which includes, for example, desquamative interstitial pneumonia, nonspecific interstitial pneumonia, lymphoid interstitial pneumonia , cryptogenic organizing pneumonia, and idiopathic pulmonary fibrosis.

[0036] "Idiopathic pulmonary fibrosis" or "IPF" as used herein refers to a chronic, progressive form of lung disease characterized by fibrosis of the supporting framework (interstitium) of the lungs. By definition, the term is used when the cause of the pulmonary fibrosis is unknown ("idiopathic"). Microscopically, lung tissue from patients having IPF

shows a characteristic set of histologic/pathologic features known as usual interstitial pneumonia (UIP), which is a pathologic counterpart of IPF.

[0037] "Nonspecific interstitial pneumonia" or "NSIP" is a form of idiopathic interstitial pneumonia generally characterized by a cellular pattern defined by chronic inflammatory cells with collagen deposition that is consistent or patchy, and a fibrosing pattern defined by a diffuse patchy fibrosis. In contrast to UIP, there is no honeycomb appearance nor fibroblast foci that characterize usual interstitial pneumonia.

[0038] "Hypersensitivity pneumonitis" or "HP" refers to also called extrinsic allergic alveolitis, (EAA) refers to an inflammation of the alveoli within the lung caused by an exaggerated immune response and hypersensitivity to as a result of an inhaled antigen (e.g., organic dust).

[0039] "Pulmonary sarcoidosis" or "PS" refers to a syndrome involving abnormal collections of chronic inflammatory cells (granulomas) that can form as nodules. The inflammatory process for HP generally involves the alveoli, small bronchi, and small blood vessels. In acute and subacute cases of HP, physical examination usually reveals dry rales.

[0040] The term "microarray" refers to an ordered arrangement of hybridizable array elements, preferably polynucleotide probes, on a substrate.

[0041] The term "polynucleotide," when used in singular or plural, generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double- stranded regions. In addition, the term "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple -helical region often is an oligonucleotide. The term "polynucleotide" can also include DNAs (e.g., cDNAs) and RNAs that contain one or more modified bases (e.g., to provide a detectable signal, such as a fluorophore). Thus, DNAs or RNAs with

backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritiated bases, are included within the term "polynucleotides" as defined herein. In general, the term "polynucleotide" embraces all chemically, enzymatically and/or metabolically modified forms of unmodified polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells.

[0042] The term "oligonucleotide" refers to a relatively short polynucleotide (e.g., 100, 50, 20 or fewer nucleotides) including, without limitation, single-stranded deoxyribonucleotides, single- or double- stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs. Oligonucleotides, such as single-stranded DNA probe oligonucleotides, are often synthesized by chemical methods, for example using automated oligonucleotide synthesizers that are commercially available. However, oligonucleotides can be made by a variety of other methods, including in vitro recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

[0043] The terms "gene product" or "expression product" are used herein interchangeably to refer to the RNA transcription products (RNA transcript) of a gene, including mRNA, and the polypeptide translation product of such RNA transcripts. A gene product can be, for example, a polynucleotide gene expression product (e.g., an unspliced RNA, an mRNA, a splice variant mRNA, a microRNA, a fragmented RNA, and the like) or a protein expression product (e.g., a mature polypeptide, a post-translationally modified polypeptide, a splice variant polypeptide, and the like). In some embodiments the gene expression product may be a sequence variant including mutations, fusions, loss of heterozygoxity (LOH), and.or biological pathway effects.

[0044] The term "normalized expression level" as applied to a gene expression product refers to a level of the gene product normalized relative to one or more reference (or control) gene expression products.

[0045] A "reference expression level" as applied to a gene expression product refers to an expression level for one or more reference (or control) gene expression products. A "reference normalized expression level" as applied to a gene expression product refers to a normalized expression level value for one or more reference (or control) gene expression products (i.e., a normalized reference expression level). In some embodiments, a reference

expression level is an expression level for one or more gene product in normal sample, as described herein. In some embodiments, a reference expression level is determined experimentally. In some embodiments, a reference expression level is a historical expression level, e.g., a database value of a reference expression level in a normal sample, which sample indicates a single reference expression level, or a summary of a plurality of reference expression levels (such as, e.g., (i) an average of two or more, preferably three or more reference expression levels from replicate analysis of the reference expression level from a single sample; (ii) an average of two or more, preferably three or more reference expression levels from analysis of the reference expression level from a plurality of different samples (e.g., normal samples); (iii) and a combination of the above mentioned steps (i) and (ii) (i.e., average of reference expression levels analyzed from a plurality of samples, wherein at least one of the reference expression levels are analyzed in replicate). In some embodiments, the "reference expression level" is an expression level of sequence variants, for example, in a sample that has been definitively determined to be UIP or non-UIP by other means (i.e. confirmed pathological diagnosis).

[0046] A "reference expression level value" as applied to a gene expression product refers to an expression level value for one or more reference (or control) gene expression products. A "reference normalized expression level value" as applied to a gene expression product refers to a normalized expression level value for one or more reference (or control) gene expression products.

[0047] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to re-anneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology,(Wiley Interscience, 1995).

[0048] "Stringent conditions" or "high stringency conditions", as defined herein, typically: (1) employ low ionic strength solutions and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

[0049] "Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press, 1989), and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent that those described above. An example of moderately stringent condition is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0050] "Sensitivity" as used herein refers to the proportion of true positives of the total number tested that actually have the target disorder (i.e., the proportion of patients with the target disorder who have a positive test result). "Specificity" as used herein refers to the proportion of true negatives of all the patients tested who actually do not have the target disorder (i.e., the proportion of patients without the target disorder who have a negative test result).

[0051] In the context of the present invention, reference to "at least one," "at least two," "at least five," etc. of the genes listed in any particular gene set means any one or any and all combinations of the genes listed.

[0052] The terms "splicing" and "RNA splicing" are used interchangeably and refer to RNA processing that removes introns and joins exons to produce mature mRNA with continuous coding sequence that moves into the cytoplasm of a eukaryotic cell.

[0053] The term "exon" refers to any segment of an interrupted gene that is represented in a mature RNA product (B. Lewin,Genes 7V(Cell Press, 1990)). In theory the term "intron" refers to any segment of DNA that is transcribed but removed from within the transcript by splicing together the exons on either side of it. Operationally, exon sequences occur in the mRNA sequence of a gene as defined by Ref. SEQ ID numbers. Operationally, intron sequences are the intervening sequences within the genomic DNA of a gene, bracketed by exon sequences and usually having GT and AG splice consensus sequences at their 5' and 3' boundaries.

[0054] A "computer-based system" refers to a system of hardware, software, and data storage medium used to analyze information. Hardware of a patient computer-based system can include a central processing unit (CPU), and hardware for data input, data output (e.g., display), and data storage. The data storage medium can include any manufacture comprising a recording of the present information as described above, or a memory access device that can access such a manufacture.

[0055] As used herein the term “module” refers to any assembly and/or set of operatively-coupled electrical components that can include, for example, a memory, a processor, electrical traces, optical connectors, software (executing in hardware), and/or the like. For example, a module executed in the processor can be any combination of hardware-based module (e.g., a field-programmable gate array (FPGA), an application specific integrated circuit (ASIC), a digital signal processor (DSP)) and/or software-based module (e.g., a module of computer code stored in memory and/or executed at the processor) capable of performing one or more specific functions associated with that module.

[0056] To "record" data, programming or other information on a computer readable medium refers to a process for storing information, using any such methods as known in the art. Any convenient data storage structure may be chosen, based on the means used to access the stored information. A variety of data processor programs and formats can be used for storage, e.g. word processing text file, database format, etc.

[0057] A "processor" or "computing means" references any hardware and/or software combination that will perform the functions required of it. For example, a suitable processor may be a programmable digital microprocessor such as available in the form of an electronic controller, mainframe, server or personal computer (desktop or portable). Where the processor is programmable, suitable programming can be communicated from a remote location to the processor, or previously saved in a computer program product (such as a portable or fixed computer readable storage medium, whether magnetic, optical or solid state device based). For example, a magnetic medium or optical disk may carry the programming, and can be read by a suitable reader communicating with each processor at its corresponding station.

[0058] A "test sample" is a sample of one or more cells, preferable a tissue sample (e.g., a lung tissue sample such as a transbronchial biopsy (TBB) sample) obtained from a subject. In some embodiments, a test sample is a biopsy sample obtained by any means known in the art. In particular embodiments, the test sample is a sample obtained by a video-assisted thoracoscopic surgery (VATS); a bronchoalveolar lavage (BAL); a transbronchial biopsy (TBB); or a cryo-transbronchial biopsy. In some embodiments the test sample is obtained from a patient suspected of having a lung disease, e.g., an ILD, based on clinical signs and symptoms with which the patient presents (e.g., shortness of breath (generally aggravated by exertion), dry cough), and, optionally the results of one or more of an imaging test (e.g., chest X-ray, computerized tomography (CT)), a pulmonary function test (e.g., spirometry, oximetry, exercise stress test), lung tissue analysis (e.g., histological and/or cytological analysis of samples obtained by bronchoscopy, bronchoalveolar lavage, surgical biopsy) .

[0059] A "gene signature" is a gene expression pattern (*i.e.*, expression level of one or more gene, or fragments thereof), which is indicative of some characteristic or phenotype. In some embodiments, gene signature refers to the expression (and/or lack of expression) of a gene, a plurality of genes, a fragment of a gene or a plurality fragments of one or more genes, which expression and/or lack of expression is indicative of UIP, Non-UIP, smoker-status, or Non-smoker-status.

[0060] As used herein, "is a smoker" is meant to refer to a subject who currently smokes cigarettes or a person who has smoked cigarettes in the past or a person who has the gene signature of a person who currently smokes cigarettes or has smoked cigarettes in the past.

[0061] As used herein, "variant", when used to describe a feature used during training of a classifier of the present invention, refers to an alternative splice variant.

[0062] As used herein, "mutation", when used to describe a feature used during training of a classifier of the present invention, refers to a sequence deviation from a known normal reference sequence. In some embodiments, the deviation is a deviation from an accepted native gene sequence according to a publically accessible database such as the UniGene database (Pontius JU, Wagner L, Schuler GD. UniGene: a unified view of the transcriptome. In: The NCBI Handbook. Bethesda (MD): National Center for Biotechnology Information; 2003, incorporated herein), RefSeq (The NCBI handbook [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project, available at the world wide web address: ncbi.nlm.nih.gov/refseq/), Ensembl (EMBL, available at the world wide web address: ensembl.org/index.html), and the like. In some embodiments, the mutation includes an addition, deletion, or substitution of a sequence residue present in the reference sequence.

[0063] Abbreviations include: HRCT, high-resolution computed tomography; VATS, video-assisted thorascopic surgery; SLB, surgical lung biopsy; TBB, transbronchial biopsy; RB, respiratory bronchiolitis; OP, organizing pneumonia, DAD, diffuse alveolar damage, CIF/NOC, chronic interstitial fibrosis not otherwise classified; MDT, multidisciplinary team; CV, cross-validation; LOPO, leave-one-patient-out; ROC, receiver operator characteristic; AUC, area under the curve; RNASeq, RNA sequencing by next-generation sequencing technology; NGS, next-generation sequencing technology; H&E, hematoxylin and eosin; FDR, false discovery rate; IRB, Institutional Review Board; ATS, American Thoracic Society; COPD, chronic obstructive pulmonary disease; KEGG, Kyoto Encyclopedia of Genes and Genomes; CI, confidence interval

[0064] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included

limits are also included in the invention. As used herein, "about" means plus or minus 10% of the indicated value.

DETAILED DESCRIPTION OF THE INVENTION

[0065] Disclosed herein are methods of and/or systems for using a molecular signature to differentiate UIP from other ILD subtypes. The accurate diagnosis of UIP from samples where expert pathology is not available stands to benefit ILD patients by accelerating diagnosis, thus facilitating treatment decisions and reducing surgical risk to patients and costs to the healthcare system.

[0066] Also disclosed herein are methods of and/or systems for using the smoker or non-smoker status of a subject to improve differentiation of UIP from other ILD subtypes using a molecular signature.

[0067] Thus, the methods and/or systems disclosed herein provide classifiers which can differentiate UIP from non-UIP patterns based on high-dimensional transcriptional data without prior knowledge of clinical or demographic information.

[0068] In some embodiments, the present invention provides methods for differentiating UIP from non-UIP using a classifier that comprises or consists of one or more sequences or fragments thereof presented in any of Tables 5, 7, 8, 9, 10, 11, or 12 or at least one sequence or fragment thereof from each of Tables 5, 7, 8, 9, 10, 11 and 12. In some embodiments, the present invention provides such methods that use a classifier comprising or consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of the sequences provided in any one or more or all of Tables 5, 7, 8, 9, 10, 11 and 12. For example, in some embodiments, the present invention provides such methods that use classifiers comprising or consisting of at least 11, 12, 13, 14, 15, 20, 30, 50, 100, 150, 200, 250, 300, or more sequences provided in any one or more or all of Tables 5, 7, 8, 9, 10, 11 and 12, including all integers (e.g., 16, 17, 18, 19, 21, 22, 23, 24, 25 sequences, etc.) and ranges (e.g., from about 1-10 sequences from any one or more or all of Tables 5, 7, 8, 9, 10, 11, and 12, from about 10-15 sequences, 10-20 sequences, 5-30 sequences, 5-50 sequences, 10-100 sequences, 50- 200 sequences, etc.) between.

[0069] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of one or more of the following sequences or fragments thereof: 1) HLA-F (SEQ ID NO.:1), 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-

G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier may contain 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes.

[0070] In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier that comprises or consists of 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; or 21 of the following sequences: 1) HLA-F (SEQ ID NO.:1), 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22) in any combination. In particular aspects, such a classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes.

[0071] In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier that comprises or consists of all of the following sequences: 1) HLA-F (SEQ ID NO.:1), 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0072] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of one or more of the following sequences or fragments thereof: 1) HLA-F (SEQ ID NO.:1), 2) HMCN2, 3) ADAMTSL1, 4) CD79B, 5) KEL, 6) KLHL14, 7) MPP2, 8) NMNAT2, 9) PLXDC1, 10) CAPN9, 11) TALDO1, 12) PLK4, 13) IGHV3-72, 14) IGKV1-9, and 15) CNTN4. In particular aspects, the classifier may contain 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes.

[0073] In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier that comprises or consists of 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; or 14 of the following sequences: 1) HLA-F (SEQ ID NO.:1), 2) HMCN2, 3) ADAMTSL1, 4) CD79B, 5) KEL, 6) KLHL14, 7) MPP2, 8) NMNAT2, 9) PLXDC1, 10) CAPN9, 11) TALDO1, 12) PLK4, 13) IGHV3-72, 14) IGKV1-9, and 15) CNTN4. In particular aspects, the classifier may contain 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes.

[0074] In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier that comprises or consists of the following sequences: 1) HLA-F (SEQ ID NO.:1), 2) HMCN2, 3) ADAMTSL1, 4) CD79B, 5) KEL, 6) KLHL14, 7) MPP2, 8) NMNAT2, 9) PLXDC1, 10) CAPN9, 11) TALDO1, 12) PLK4, 13) IGHV3-72, 14) IGKV1-9, and 15) CNTN4. In particular aspects, the classifier may contain 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes.

[0075] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of HLA-F (SEQ ID NO.:1) or fragments thereof. In one such embodiment, the method uses a classifier comprising 1) HLA-F (SEQ ID NO.:1) and at least one of 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4

(SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0076] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of HMCN2 or fragments thereof. In one such embodiment, the method uses a classifier comprising HMCN2 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0077] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of ADAMTSL1 or fragments thereof. In one such embodiment, the method uses a classifier comprising ADAMTSL1 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0078] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of CD79B or fragments thereof. In one such embodiment, the method uses a classifier comprising CD79B and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0079] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of KEL or fragments thereof. In one such embodiment, the method uses a classifier comprising KEL and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0080] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of KLHL14 or fragments thereof. In one such embodiment, the method uses a classifier comprising KLHL14 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4

(SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0081] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of MPP2 or fragments thereof. In one such embodiment, the method uses a classifier comprising MPP2 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0082] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of NMNAT2 or fragments thereof. In one such embodiment, the method uses a classifier comprising NMNAT2 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0083] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of PLXDC1 or fragments thereof. In one such embodiment, the method uses a classifier comprising PLXDC1 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0084] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of CAPN9 or fragments thereof. In one such embodiment, the method uses a classifier comprising CAPN9 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0085] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of TALDO1 or fragments thereof. In one such embodiment, the method uses a classifier comprising TALDO1 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ

ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0086] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of PLK4 or fragments thereof. In one such embodiment, the method uses a classifier comprising PLK4 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0087] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of IGHV3-72 or fragments thereof. In one such embodiment, the method uses a classifier comprising IGHV3-72 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0088] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of IGKV1-9 or fragments thereof. In one such embodiment, the method uses a classifier comprising IGKV1-9 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0089] In some particular embodiments, the present invention provides methods and/or systems for differentiating UIP from non-UIP using a classifier that comprises or consists of CNTN4 or fragments thereof. In one such embodiment, the method uses a classifier comprising CNTN4 and at least one of 1) HLA-F (SEQ ID NO.:1) 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes.

[0090] In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier that comprises or consists of 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 of the following sequences: 1) HLA-F (SEQ ID NO.:1), 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ

ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), 22) DES (SEQ ID NO.:22), 23) HMCN2, 24) ADAMTSL1, 25) CD79B, 26) KEL, 27) KLHL14, 28) MPP2, 29) NMNAT2, 30) PLXDC1, 31) CAPN9, 32) TALDO1, 33) PLK4, 34) IGHV3-72, 35) IGKV1-9, and 36) CNTN4. In particular aspects, the classifier may contain 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes.

[0091] In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier that comprises or consists of all of the following sequences: 1) HLA-F (SEQ ID NO.:1), 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), 22) DES (SEQ ID NO.:22), 23) HMCN2, 24) ADAMTSL1, 25) CD79B, 26) KEL, 27) KLHL14, 28) MPP2, 29) NMNAT2, 30) PLXDC1, 31) CAPN9, 32) TALDO1, 33) PLK4, 34) IGHV3-72, 35) IGKV1-9, and 36) CNTN4. In particular aspects, the classifier may contain 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes. In other aspects, the classifier may omit 1, 2, 3, 4, 5, 6, 7, 8, or more, of these genes, while optionally including other genes. In some embodiments, the present invention provides a method and/or system for differentiating UIP from non-UIP using a classifier described herein, wherein the method further comprises implementing a classifier that classifies the subject as a smoker or non-smoker. Such a smoker status classification can optionally be implemented prior to implementing a UIP vs. Non-UIP classifier, or a smoker status classification step can be built in as a covariate used during the training (e.g., using a classifier training module) of a UIP vs. Non-UIP classifier of the present invention.

[0092] In some embodiments, alternatively, or additionally, the method of and/or system for differentiating UIP from non-UIP using a classifier described herein further comprises a step of excluding or assigning differential weight to certain genes or variants thereof that are

susceptible to smoker-status bias during the training (e.g., using a classifier training module) or implementation of the UIP vs. Non-UIP classifier. As used herein, "smoker status bias" refers to genes or variants thereof, which in non-smoker patients are differentially expressed in UIP vs. non-UIP patients, but which are not detectably differentially expressed in UIP vs. non-UIP patients that are (or have been) smokers.

[0093] In some embodiments, the method of and/or system for the present invention comprises a tiered classifier comprising at least a first and a second classifier, wherein the first classifier is trained (e.g., using a classifier training module) to recognize gene signatures that distinguish smokers from non-smokers, and a second classifier is trained (e.g., using a classifier training module) to distinguish UIP vs. Non UIP in smokers or non-smokers, respectively.

[0094] In some embodiments, the method and/or systems of the present invention comprises:

extracting nucleic acids (e.g., RNA, such as, e.g., total RNA) from a test sample (e.g. lung tissue);

amplifying the nucleic acid to produce an expressed nucleic acid library (e.g., via polymerase chain reaction-mediated amplification of cDNAs (optionally labeled cDNAs), which cDNAs may be produced from one or more RNA sample by reverse transcription (RT-PCR));

detecting expression of one or more nucleic acid present in the nucleic acid library (e.g., detecting RNA expression profiles by measuring cDNA species produced via RT-PCR) via an array (e.g., a microarray) or via direct sequencing (e.g., RNAseq); and

determining whether the test sample is UIP or non-UIP using a trained classifier described herein.

[0095] In some embodiments, the method and/or system of the present invention further comprises incorporating smoker status into the training exercise. In certain embodiments, smoker status is optionally incorporated in one of the following ways:

(i) by using smoking status as a covariate in a UIP or Non-UIP classifier during training (e.g., using a classifier training module).

(ii) by identifying a plurality of genes that are susceptible to smoker-status bias and excluding, or optionally weighing such genes differently than genes that are not susceptible to such bias, during UIP or Non-UIP classifier training (e.g., using a classifier training module).

(iii) by constructing a tiered classification in which an initial classifier that is trained (e.g., using a classifier training module) to recognize gene signatures that distinguish smokers from non-smokers is used to pre-classify a test sample as "smoker" or "non-smoker" based upon the gene signature of the test sample; and then, subsequent to pre-classification, a distinct classifier that was trained (e.g., using a classifier training module) to distinguish UIP vs. Non UIP in either smokers or non-smokers is implemented. For example, if the pre-classifier determines that the test sample is from a smoker, a UIP vs. Non-UIP classification is performed using a classifier trained (e.g., using a classifier training module) with UIP and Non-UIP samples from smokers. Conversely, if the pre-classifier determines that the test sample is from a non-smoker, a UIP vs. Non-UIP classification is performed using a classifier trained (e.g., using a classifier training module) with UIP and Non-UIP samples from non-smokers. In some embodiments, such smoker- or non-smoker-specific classifiers provide improved diagnostic performance due, at least in part, to a reduction in background noise caused by the inclusion of genes susceptible to smoker-status bias in the classifier training.

[0096] Accordingly, the present invention also provides suitable classifiers for use in methods of differentiating UIP from non-UIP, as disclosed herein. In various embodiments, the present invention provides a classifier suitable for differentiating UIP from non-UIP, wherein the classifier is trained (e.g., using a classifier training module) using microarray or sequencing data from a sample corresponding to one or more histopathology label determined by an expert pathologist. In some embodiments, the sample is labelled UIP or Non-UIP.

[0097] In some embodiments, the present invention presents a classifier comprising or consisting of one or more sequences or fragments thereof presented in any of Tables 5, 7, 8, 9, 10, 11, or 12, or at least one sequence or fragment thereof from each of Tables 5, 7, 8, 9, 10, 11, or 12. In some embodiments, the present invention provides a classifier comprising or consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of the sequences provided in any one or more or all of Tables 5, 7, 8, 9, 10, 11 and 12. For example, in some embodiments, the present invention provides a classifier comprising or consisting of at least 11, 12, 13, 14, 15, 20, 30, 50, 100, 150, 200, 250, 300, or more sequences provided in any one or more or all of Tables 5, 7, 8, 9, 10, 11, or 12, including all integers (e.g., 16, 17, 18, 19, 21, 22, 23, 24, 25 sequences, etc.) and ranges (e.g., from about 1-10 sequences from any one or more or all of Tables 5, 7, 8, 9, 10, 11, or 12, from about 10-15 sequences, 10-20 sequences, 5-30 sequences, 5-50 sequences, 10-100 sequences, 50- 200 sequences from any one or more or all of Tables 5, 7, 8, 9, 10, 11, or 12, etc.) between. In one embodiment, the present invention

provides a classifier that comprises or consists of all sequences provided in Table 5, all sequences provided in Table 7, all sequences provided in Table 8, all sequences provided in Table 9, all sequences provided in table 10, all sequences provided in Table 11, or all sequences provided in Table 12. In one embodiment, the present invention provides a classifier that comprises or consists of all sequences provided in each of Tables 5, 7, 8, 9, 10, 11, or 12.

[0098] In some particular embodiments, the present invention provides a classifier for differentiating UIP from non-UIP, wherein the classifier comprises or consists of one or more of the following sequences or fragments thereof: 1) HLA-F (SEQ ID NO.:1), 2) CDKL2 (SEQ ID NO.:2), 3) GPR98 (SEQ ID NO.:3), 4) PRKCQ (SEQ ID NO.:4), 5) HLA-G (SEQ ID NO.:5), 6) PFKFB3 (SEQ ID NO.:6), 7) CEACAM1 (SEQ ID NO.:7), 8) RABGAP1L (SEQ ID NO.:8), 9) CD274 (SEQ ID NO.:9), 10) PRUNE2 (SEQ ID NO.:10), 11) ARAP2 (SEQ ID NO.:11), 12) DZIP1 (SEQ ID NO.:12), 13) MXRA7 (SEQ ID NO.:13), 14) PTCHD4 (SEQ ID NO.:14), 15) PDLIM3 (SEQ ID NO.:15), 16) CNN1 (SEQ ID NO.:16), 17) NIPSNAP3B (SEQ ID NO.:17), 18) PAQR7 (SEQ ID NO.:18), 19) ACTG2 (SEQ ID NO.:19), 20) NA (SEQ ID NO.:20), 21) TIMP2 (SEQ ID NO.:21), and 22) DES (SEQ ID NO.:22). In one embodiment, the classifier comprises or consists of all 22 of the above mentioned sequences. In some embodiments, the present invention provides a classifier for differentiating UIP from non-UIP, wherein the classifier comprises or consists of 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; or 21 of the abovementioned 22 sequences. In particular aspects, the classifier contains 1, 2, 3, 4, 5, 6, 7, 8, or more additional genes or fragments thereof. In other aspects, the classifier omits 1, 2, 3, 4, 5, 6, 7, 8, or more, of the abovementioned 22 sequences, while optionally including other genes. In other aspects, each of the 22 genes may be used in combination with any 1 or more, up to 20 more, of the other genes.

Tissue samples

[0099] A lung tissue sample for use in a subject analytical or diagnostic method can be a biopsy sample (e.g., a biopsy sample obtained by video-assisted thoracoscopic surgery; VATS); a bronchoalveolar lavage (BAL) sample; a transbronchial biopsy; a cryo-transbronchial biopsy; and the like." Lung tissue samples for analysis can be provided in a suitable preservation solution.

[00100] Tissue samples can be obtained from a patient suspected of having a lung disease, e.g., an ILD, based on clinical signs and symptoms with which the patient presents (e.g., shortness of breath (generally aggravated by exertion), dry cough), and, optionally the results of one or more of an imaging test (e.g., chest X-ray, computerized tomography (CT)), a pulmonary function test (e.g., spirometry, oximetry, exercise stress test), lung tissue analysis (e.g., histological and/or cytological analysis of samples obtained by bronchoscopy, bronchoalveolar lavage, surgical biopsy) .

[00101] The lung tissue sample can be processed in any of a variety of ways. For example, the lung tissue sample can be subjected to cell lysis. The lung tissue sample can be preserved in RNAprotect solution (a solution that inhibits RNA degradation, e.g., that inhibits nuclease digestion of RNA) and subsequently subjected to cell lysis. Components such as nucleic acids and/or proteins can be enriched or isolated from the lung tissue sample, and the enriched or isolated component can be used in a subject method. Methods of enriching for and isolating components such nucleic acids and proteins are known in the art; and any known method can be used. Methods of isolating RNA for expression analysis have been described in the art.

In vitro methods of determining expression product levels

[00102] Additional approaches to assess expression of the panel further demonstrated the genomic signal observed in UIP vs. non-UIP classification is robust across diverse biochemical assays and detection methods. Specifically we generated RNASeq data for a subset of the cohort and evaluated performance under CV. Performance comparisons with matched array data demonstrated that classification using RNASeq data achieves similar performance to data generated from the microarray platform.

[00103] The general methods for determining gene expression product levels are known to the art and may include but are not limited to one or more of the following: additional cytological assays, assays for specific proteins or enzyme activities, assays for specific expression products including protein or RNA or specific RNA splice variants, in situ hybridization, whole or partial genome expression analysis, microarray hybridization assays, serial analysis of gene expression (SAGE), enzyme linked immunoabsorbance assays, mass-spectrometry, immunohistochemistry, blotting, sequencing, RNA sequencing, DNA sequencing (e.g., sequencing of cDNA obtained from RNA); Next-Gen sequencing, nanopore sequencing, pyrosequencing, or Nanostring sequencing. For example, gene expression

product levels can be determined according to the methods described in Kim, et.al. (Lancet Respir Med. 2015 Jun;3(6):473-82, incorporated herein in its entirety, including all supplements). As used herein, the terms "assaying" or "detecting" or "determining" are used interchangeably in reference to determining gene expression product levels, and in each case, it is contemplated that the above-mentioned methods of determining gene expression product levels are suitable for detecting or assaying gene expression product levels. Gene expression product levels may be normalized to an internal standard such as total mRNA or the expression level of a particular gene including but not limited to glyceraldehyde 3 phosphate dehydrogenase, or tubulin.

[00104] In various embodiments, a sample comprises cells harvested from a tissue sample (e.g., a lung tissue sample such as a TBB sample). Cells can be harvested from a sample using standard techniques known in the art or disclosed herein. For example, in one embodiment, cells are harvested by centrifuging a cell sample and resuspending the pelleted cells. The cells can be resuspended in a buffered solution such as phosphate-buffered saline (PBS). After centrifuging the cell suspension to obtain a cell pellet, the cells can be lysed to extract nucleic acid, *e.g*, messenger RNA. All samples obtained from a subject, including those subjected to any sort of further processing, are considered to be obtained from the subject.

[00105] The sample, in one embodiment, is further processed before detection of the gene expression products is performed as described herein. For example, mRNA in a cell or tissue sample can be separated from other components of the sample. The sample can be concentrated and/or purified to isolate mRNA in its non-natural state, as the mRNA is not in its natural environment. For example, studies have indicated that the higher order structure of mRNA *in vivo* differs from the *in vitro* structure of the same sequence (*see, e.g.*, Rouskin *et al.* (2014). Nature 505, pp. 701-705, incorporated herein in its entirety for all purposes).

[00106] mRNA from the sample in one embodiment, is hybridized to a synthetic DNA probe, which in some embodiments, includes a detection moiety (e.g., detectable label, capture sequence, barcode reporting sequence). Accordingly, in these embodiments, a non-natural mRNA-cDNA complex is ultimately made and used for detection of the gene expression product. In another embodiment, mRNA from the sample is directly labeled with a detectable label, *e.g.*, a fluorophore. In a further embodiment, the non-natural labeled-mRNA molecule is hybridized to a cDNA probe and the complex is detected.

[00107] In one embodiment, once the mRNA is obtained from a sample, it is converted to complementary DNA (cDNA) in a hybridization reaction or is used in a hybridization reaction together with one or more cDNA probes. cDNA does not exist *in vivo* and therefore is a non-natural molecule. Furthermore, cDNA-mRNA hybrids are synthetic and do not exist *in vivo*. Besides cDNA not existing *in vivo*, cDNA is necessarily different than mRNA, as it includes deoxyribonucleic acid and not ribonucleic acid. The cDNA is then amplified, for example, by the polymerase chain reaction (PCR) or other amplification method known to those of ordinary skill in the art. For example, other amplification methods that may be employed include the ligase chain reaction (LCR) (Wu and Wallace, Genomics, 4:560 (1989), Landegren *et al.*, Science, 241:1077 (1988), incorporated by reference in its entirety for all purposes, transcription amplification (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:1173 (1989), incorporated by reference in its entirety for all purposes), self-sustained sequence replication (Guatelli *et al.*, *Proc. Nat. Acad. Sci. USA*, 87:1874 (1990), incorporated by reference in its entirety for all purposes), incorporated by reference in its entirety for all purposes, and nucleic acid based sequence amplification (NASBA). Guidelines for selecting primers for PCR amplification are known to those of ordinary skill in the art. *See, e.g.*, McPherson et al., PCR Basics: From Background to Bench, Springer-Verlag, 2000, incorporated by reference in its entirety for all purposes. The product of this amplification reaction, *i.e.*, amplified cDNA is also necessarily a non-natural product. First, as mentioned above, cDNA is a non-natural molecule. Second, in the case of PCR, the amplification process serves to create hundreds of millions of cDNA copies for every individual cDNA molecule of starting material. The number of copies generated are far removed from the number of copies of mRNA that are present *in vivo*.

[00108] In one embodiment, cDNA is amplified with primers that introduce an additional DNA sequence (*e.g.*, adapter, reporter, capture sequence or moiety, barcode) onto the fragments (*e.g.*, with the use of adapter-specific primers), or mRNA or cDNA gene expression product sequences are hybridized directly to a cDNA probe comprising the additional sequence (e.g., adapter, reporter, capture sequence or moiety, barcode). Amplification and/or hybridization of mRNA to a cDNA probe therefore serves to create non-natural double stranded molecules from the non-natural single stranded cDNA, or the mRNA, by introducing additional sequences and forming non-natural hybrids. Further, as known to those of ordinary skill in the art, amplification procedures have error rates associated with them. Therefore, amplification introduces further modifications into the

cDNA molecules. In one embodiment, during amplification with the adapter-specific primers, a detectable label, *e.g.*, a fluorophore, is added to single strand cDNA molecules. Amplification therefore also serves to create DNA complexes that do not occur in nature, at least because (i) cDNA does not exist *in vivo*, (i) adapter sequences are added to the ends of cDNA molecules to make DNA sequences that do not exist *in vivo*, (ii) the error rate associated with amplification further creates DNA sequences that do not exist *in vivo*, (iii) the disparate structure of the cDNA molecules as compared to what exists in nature and (iv) the chemical addition of a detectable label to the cDNA molecules.

[00109] In some embodiments, the expression of a gene expression product of interest is detected at the nucleic acid level via detection of non-natural cDNA molecules.

[00110] The gene expression products described herein include RNA comprising the entire or partial sequence of any of the nucleic acid sequences of interest, or their non-natural cDNA product, obtained synthetically *in vitro* in a reverse transcription reaction. The term "fragment" is intended to refer to a portion of the polynucleotide that generally comprise at least 10, 15, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1,000, 1,200, or 1,500 contiguous nucleotides, or up to the number of nucleotides present in a fulllength gene expression product polynucleotide disclosed herein. A fragment of a gene expression product polynucleotide will generally encode at least 15, 25, 30, 50, 100, 150, 200, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length gene expression product protein of the invention.

[00111] In certain embodiments, a gene expression profile may be obtained by whole transcriptome shotgun sequencing ("WTSS" or "RNAseq"; see, e.g., Ryan et al BioTechniques 45: 81- 94), which makes the use of high-throughput sequencing technologies to sequence cDNA in order to about information about a sample's RNA content. In general terms, cDNA is made from RNA, the cDNA is amplified, and the amplification products are sequenced.

[00112] After amplification, the cDNA may be sequenced using any convenient method. For example, the fragments may be sequenced using Illumina's reversible terminator method, Roche's pyrosequencing method (454), Life Technologies' sequencing by ligation (the SOLiD platform) or Life Technologies' Ion Torrent platform. Examples of such methods are described in the following references: Margulies et al (Nature 2005 437: 376-80); Ronaghi et al (Analytical Biochemistry 1996 242: 84-9); Shendure (Science 2005 309: 1728);

Imelfort et al (Brief Bioinform. 2009 10:609-18); Fox et al (Methods Mol Biol. 2009;553:79-108); Appleby et al (Methods Mol Biol. 2009;513: 19-39) and Morozova (Genomics. 2008 92:255-64), which are incorporated by reference for the general descriptions of the methods and the particular steps of the methods, including all starting products, reagents, and final products for each of the steps. As would be apparent, forward and reverse sequencing primer sites that compatible with a selected next generation sequencing platform can be added to the ends of the fragments during the amplification step.

[00113] In other embodiments, the products may be sequenced using nanopore sequencing (e.g. as described in Soni et al Clin Chem 53: 1996-2001 2007, or as described by Oxford Nanopore Technologies). Nanopore sequencing is a single-molecule sequencing technology whereby a single molecule of DNA is sequenced directly as it passes through a nanopore. A nanopore is a small hole, of the order of 1 nanometer in diameter. Immersion of a nanopore in a conducting fluid and application of a potential (voltage) across it results in a slight electrical current due to conduction of ions through the nanopore. The amount of current which flows is sensitive to the size and shape of the nanopore. As a DNA molecule passes through a nanopore, each nucleotide on the DNA molecule obstructs the nanopore to a different degree, changing the magnitude of the current through the nanopore in different degrees. Thus, this change in the current as the DNA molecule passes through the nanopore represents a reading of the DNA sequence. Nanopore sequencing technology as disclosed in U.S. Pat. Nos. 5,795,782, 6,015,714, 6,627,067, 7,238,485 and 7,258,838 and U.S. patent application publications US2006003171 and US20090029477.

[00114] In some embodiments, the gene expression product of the subject methods is a protein, and the amount of protein in a particular biological sample is analyzed using a classifier derived from protein data obtained from cohorts of samples. The amount of protein can be determined by one or more of the following: enzyme-linked immunosorbent assay (ELISA), mass spectrometry, blotting, or immunohistochemistry.

[00115] In some embodiments, gene expression product markers and alternative splicing markers may be determined by microarray analysis using, for example, Affymetrix arrays, cDNA microarrays, oligonucleotide microarrays, spotted microarrays, or other microarray products from Biorad, Agilent, or Eppendorf. Microarrays provide particular advantages because they may contain a large number of genes or alternative splice variants that may be assayed in a single experiment. In some cases, the microarray device may contain

the entire human genome or transcriptome or a substantial fraction thereof allowing a comprehensive evaluation of gene expression patterns, genomic sequence, or alternative splicing. Markers may be found using standard molecular biology and microarray analysis techniques as described in Sambrook Molecular Cloning a Laboratory Manual 2001 and Baldi, P., and Hatfield, W. G., DNA Microarrays and Gene Expression 2002.

[00116] Microarray analysis generally begins with extracting and purifying nucleic acid from a biological sample, (e.g. a biopsy or fine needle aspirate) using methods known to the art. For expression and alternative splicing analysis it may be advantageous to extract and/or purify RNA from DNA. It may further be advantageous to extract and/or purify niRNA from other forms of RNA such as tRNA and rRNA.

[00117] Purified nucleic acid may further be labeled with a fluorescent label, radionuclide, or chemical label such as biotin, digoxigenin, or digoxin for example by reverse transcription, polymerase chain reaction (PCR), ligation, chemical reaction or other techniques. The labeling can be direct or indirect which may further require a coupling stage. The coupling stage can occur before hybridization, for example, using aminoallyl-UTP and NHS amino-reactive dyes (like cyanine dyes) or after, for example, using biotin and labelled streptavidin. In one example, modified nucleotides (e.g. at a 1 aaUTP: 4 TTP ratio) are added enzymatically at a lower rate compared to normal nucleotides, typically resulting in 1 every 60 bases (measured with a spectrophotometer). The aaDNA may then be purified with, for example, a column or a diafiltration device. The aminoallyl group is an amine group on a long linker attached to the nucleobase, which reacts with a reactive label (e.g. a fluorescent dye).

[00118] The labeled samples may then be mixed with a hybridization solution which may contain sodium dodecyl sulfate (SDS), SSC, dextran sulfate, a blocking agent (such as COT1 DNA, salmon sperm DNA, calf thymus DNA, PolyA or PolyT), Denhardt's solution, formamine, or a combination thereof.

[00119] A hybridization probe is a fragment of DNA or RNA of variable length, which is used to detect in DNA or RNA samples the presence of nucleotide sequences (the DNA target) that are complementary to the sequence in the probe. The probe thereby hybridizes to single-stranded nucleic acid (DNA or RNA) whose base sequence allows probe-target base pairing due to complementarity between the probe and target. The labeled probe is first

denatured (by heating or under alkaline conditions) into single DNA strands and then hybridized to the target DNA.

[00120] To detect hybridization of the probe to its target sequence, the probe is tagged (or labeled) with a molecular marker; commonly used markers are 32P or Digoxigenin, which is nonradioactive antibody-based marker. DNA sequences or RNA transcripts that have moderate to high sequence complementarity (e.g. at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more complementarity) to the probe are then detected by visualizing the hybridized probe via autoradiography or other imaging techniques. Detection of sequences with moderate or high complementarity depends on how stringent the hybridization conditions were applied; high stringency, such as high hybridization temperature and low salt in hybridization buffers, permits only hybridization between nucleic acid sequences that are highly similar, whereas low stringency, such as lower temperature and high salt, allows hybridization when the sequences are less similar. Hybridization probes used in DNA microarrays refer to DNA covalently attached to an inert surface, such as coated glass slides or gene chips, and to which a mobile cDNA target is hybridized.

[00121] A mix comprising target nucleic acid to be hybridized to probes on an array may be denatured by heat or chemical means and added to a port in a microarray. The holes may then be sealed and the microarray hybridized, for example, in a hybridization oven, where the microarray is mixed by rotation, or in a mixer. After an overnight hybridization, non-specific binding may be washed off (e.g. with SDS and SSC). The microarray may then be dried and scanned in a machine comprising a laser that excites the dye and a detector that measures emission by the dye. The image may be overlaid with a template grid and the intensities of the features (e.g. a feature comprising several pixels) may be quantified.

[00122] Various kits can be used for the amplification of nucleic acid and probe generation of the subject methods. Examples of kit that can be used in the present invention include but are not limited to Nugen WT-Ovation FFPE kit, cDNA amplification kit with Nugen Exon Module and Frag/Label module. The NuGEN WT-Ovation™. FFPE System V2 is a whole transcriptome amplification system that enables conducting global gene expression analysis on the vast archives of small and degraded RNA derived from FFPE samples. The system is comprised of reagents and a protocol required for amplification of as little as 50 ng of total FFPE RNA. The protocol can be used for qPCR, sample archiving, fragmentation, and labeling. The amplified cDNA can be fragmented and labeled in less than two hours for

GeneChip™. 3' expression array analysis using NuGEN's FL-Ovation™. cDNA Biotin Module V2. For analysis using Affymetrix GeneChip™. Exon and Gene ST arrays, the amplified cDNA can be used with the WT- Ovation Exon Module, then fragmented and labeled using the FL-Ovation™. cDNA Biotin Module V2. For analysis on Agilent arrays, the amplified cDNA can be fragmented and labeled using NuGEN's FL- Ovation™. cDNA Fluorescent Module.

[00123] In some embodiments, Ambion WT -expression kit can be used. Ambion WT-expression kit allows amplification of total RNA directly without a separate ribosomal RNA (rRNA) depletion step. With the Ambion™ WT Expression Kit, samples as small as 50 ng of total RNA can be analyzed on Affymetrix™. GeneChip™ Human, Mouse, and Rat Exon and Gene 1.0 ST Arrays. In addition to the lower input RNA requirement and high concordance between the Affymetrix™ method and TaqMan™ real-time PCR data, the Ambion™. WT Expression Kit provides a significant increase in sensitivity. For example, a greater number of probe sets detected above background can be obtained at the exon level with the Ambion™. WT Expression Kit as a result of an increased signal-to-noise ratio. Ambion™-expression kit may be used in combination with additional Affymetrix labeling kit. In some embodiments, AmpTec Trinucleotide Nano mRNA Amplification kit (6299-A15) can be used in the subject methods. The ExpressArt™ TRinucleotide mRNA amplification Nano kit is suitable for a wide range, from 1 ng to 700 ng of input total RNA. According to the amount of input total RNA and the required yields of aRNA, it can be used for 1-round (input >300 ng total RNA) or 2-rounds (minimal input amount 1 ng total RNA), with aRNA yields in the range of >10 μg. AmpTec's proprietary TRinucleotide priming technology results in preferential amplification of mRNAs (independent of the universal eukaryotic 3'-poly(A)-sequence), combined with selection against rRNAs. More information on AmpTec Trinucleotide Nano mRNA Amplification kit can be obtained at www.amp-tec.com/products.htm. This kit can be used in combination with cDNA conversion kit and Affymetrix labeling kit.

[00124] The raw data may then be normalized, for example, by subtracting the background intensity and then dividing the intensities making either the total intensity of the features on each channel equal or the intensities of a reference gene and then the t-value for all the intensities may be calculated. More sophisticated methods, include z-ratio, loess and lowess regression and RMA (robust multichip analysis), such as for Affymetrix chips.

[00125] In some embodiments, the above described methods may be used for determining transcript expression levels for training (e.g., using a classifier training module) a classifier to differentiate whether a subject is a smoker or non-smoker. In some embodiments, the above described methods may be used for determining transcript expression levels for training (e.g., using a classifier training module) a classifier to differentiate whether a subject has UIP or non-UIP.

DATA ANALYSIS

(i) Comparison of Sample to Normal

[00126] In some embodiments, results of molecular profiling performed on a sample from a subject ("test sample") may be compared to a biological sample that is known or suspected to be normal ("normal sample"). In some embodiments, a normal sample is a sample that does not comprise or is expected to not comprise an ILD, or conditions under evaluation, or would test negative in the molecular profiling assay for the one or more ILDs under evaluation. In some embodiments, a normal sample is that which is or is expected to be free of any ILD, or a sample that would test negative for any ILD in the molecular profiling assay. The normal sample may be from a different subject from the subject being tested, or from the same subject. In some cases, the normal sample is a lung tissue sample obtained from a subject such as the subject being tested for example. The normal sample may be assayed at the same time, or at a different time from the test sample. In some embodiments, a normal sample is a sample that is known or suspected to be from a non-smoker. In particular embodiments, the normal sample is a sample that has been confirmed by at least two expert pathologists to be Non-UIP. In particular embodiments, the normal sample is a sample that has been confirmed by at least two expert pathologists to be Non-IPF.

[00127] The results of an assay on the test sample may be compared to the results of the same assay on a sample having a known disease state (e.g., normal, affected by a selected ILD (e.g., IPF, NSIP, etc.), smoker, non-smoker). In some cases the results of the assay on the normal sample are from a database, or a reference. In some cases, the results of the assay on the normal sample are a known or generally accepted value or range of values by those skilled in the art. In some cases the comparison is qualitative. In other cases the comparison is quantitative. In some cases, qualitative or quantitative comparisons may involve but are not limited to one or more of the following: comparing fluorescence values, spot intensities, absorbance values, chemiluminescent signals, histograms, critical threshold values, statistical

significance values, gene product expression levels, gene product expression level changes, alternative exon usage, changes in alternative exon usage, protein levels, DNA polymorphisms, copy number variations, indications of the presence or absence of one or more DNA markers or regions, or nucleic acid sequences.

(ii) Evaluation of Results

[00128] In some embodiments, the molecular profiling results are evaluated using methods known to the art for correlating gene product expression levels or alternative exon usage with specific phenotypes such as a particular ILD, or normalcy (e.g. disease or condition free). In some cases, a specified statistical confidence level may be determined in order to provide a diagnostic confidence level. For example, it may be determined that a confidence level of greater than 90% may be a useful predictor of the presence of an ILD or of a smoker or non-smoker status. In other embodiments, more or less stringent confidence levels may be chosen. For example, a confidence level of about or at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, 99.5%, or 99.9% may be chosen as a useful phenotypic predictor. The confidence level provided may in some cases be related to the quality of the sample, the quality of the data, the quality of the analysis, the specific methods used, and/or the number of gene expression products analyzed. The specified confidence level for providing a diagnosis may be chosen on the basis of the expected number of false positives or false negatives and/or cost. Methods for choosing parameters for achieving a specified confidence level or for identifying markers with diagnostic power include but are not limited to Receiver Operating Characteristic (ROC) curve analysis, binormal ROC, principal component analysis, partial least squares analysis, singular value decomposition, least absolute shrinkage and selection operator analysis, least angle regression, and the threshold gradient directed regularization method.

(iii) Data analysis

[00129] Raw gene expression level and alternative splicing data may in some cases be improved through the application of methods and/or processes designed to normalize and or improve the reliability of the data. In some embodiments of the present disclosure the data analysis requires a computer or other device, machine or apparatus for application of the various methods and/or processes described herein due to the large number of individual data points that are processed. A "machine learning classifier" refers to a computational- based prediction data structure or method, employed for characterizing a gene expression profile.

The signals corresponding to certain expression levels, which are obtained by, e.g., microarray-based hybridization assays, are typically subjected to the classifier to classify the expression profile. Supervised learning generally involves "training" a classifier to recognize the distinctions among classes and then "testing" the accuracy of the classifier on an independent test set. For new, unknown samples the classifier can be used to predict the class in which the samples belong. In various embodiments, such training is be achieved, e.g., using a classifier training module.

[00130] In some cases, the robust multi-array average (RMA) method may be used to normalize raw data. The RMA method begins by computing background-corrected intensities for each matched cell on a number of microarrays. The background corrected values are restricted to positive values as described by Irizarry et al. Biostatistics 2003 April 4 (2): 249-64. After background correction, the base-2 logarithm of each background corrected matched-cell intensity is then obtained. The back-ground corrected, log-transformed, matched intensity on each microarray is then normalized using the quantile normalization method in which for each input array and each probe expression value, the array percentile probe value is replaced with the average of all array percentile points, this method is more completely described by Bolstad et al. Bioinformatics 2003. Following quantile normalization, the normalized data may then be fit to a linear model to obtain an expression measure for each probe on each microarray. Tukey's median polish algorithm (Tukey, J. W., Exploratory Data Analysis. 1977) may then be used to determine the log-scale expression level for the normalized probe set data.

[00131] Various other software and/or hardware modules or processes may be implemented. In certain methods, feature selection and model estimation may be performed by logistic regression with *lasso* penalty using *glmnet (*Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *Journal of statistical software* 2010; **33**(1): 1-22). Raw reads may be aligned using TopHat (Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 2009; **25**(9): 1105-11.). Gene counts may be obtained using HTSeq (Anders S, Pyl PT, Huber W. HTSeq-a Python framework to work with high-throughput sequencing data. *Bioinformatics* 2014.) and normalized using DESeq (Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2; 2014). In methods, top features (N ranging from 10 to 200) were used to train a linear support vector machine (SVM) (Suykens JAK, Vandewalle J. Least Squares Support Vector Machine

Classifiers. *Neural Processing Letters* 1999; **9**(3): 293-300) using the *e1071* library (Meyer D. Support vector machines: the interface to libsvm in package e1071. 2014.). Confidence intervals may be computed using the pROC package (Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC bioinformatics* 2011; **12**: 77)

[00132] In addition, data may be filtered to remove data that may be considered suspect. In some embodiments, data deriving from microarray probes that have fewer than about 4, 5, 6, 7 or 8 guanosine+cytosine nucleotides may be considered to be unreliable due to their aberrant hybridization propensity or secondary structure issues. Similarly, data deriving from microarray probes that have more than about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 guanosine+cytosine nucleotides may be considered unreliable due to their aberrant hybridization propensity or secondary structure issues.

[00133] In some cases, unreliable probe sets may be selected for exclusion from data analysis by ranking probe-set reliability against a series of reference datasets. For example, RefSeq or Ensembl (EMBL) are considered very high quality reference datasets. Data from probe sets matching RefSeq or Ensembl sequences may in some cases be specifically included in microarray analysis experiments due to their expected high reliability. Similarly data from probe-sets matching less reliable reference datasets may be excluded from further analysis, or considered on a case by case basis for inclusion. In some cases, the Ensembl high throughput cDNA (HTC) and/or mRNA reference datasets may be used to determine the probe-set reliability separately or together. In other cases, probe-set reliability may be ranked. For example, probes and/or probe-sets that match perfectly to all reference datasets such as for example RefSeq, HTC, HTSeq, and mRNA, may be ranked as most reliable (1). Furthermore, probes and/or probe-sets that match two out of three reference datasets may be ranked as next most reliable (2), probes and/or probe- sets that match one out of three reference datasets may be ranked next (3) and probes and/or probe sets that match no reference datasets may be ranked last (4). Probes and or probe-sets may then be included or excluded from analysis based on their ranking. For example, one may choose to include data from category 1, 2, 3, and 4 probe-sets; category 1, 2, and 3 probe-sets; category 1 and 2 probe-sets; or category 1 probe-sets for further analysis. In another example, probe-sets may be ranked by the number of base pair mismatches to reference dataset entries. It is understood that there are many methods understood in the art for assessing the reliability of a given probe

and/or probe-set for molecular profiling and the methods of the present disclosure encompass any of these methods and combinations thereof..

[00134] In some embodiments of the present invention, data from probe-sets may be excluded from analysis if they are not expressed or expressed at an undetectable level (not above background). A probe-set is judged to be expressed above background if for any group:

Integral from TO to Infinity of the standard normal distribution<Significance (0.01)

Where: T0=Sqr(GroupSize) (T-P)/Sqr(Pvar); GroupSize=Number of CEL files in the group, T=Average of probe scores in probe-set, P=Average of Background probes averages of GC content, and Pvar=Sum of Background probe variances/(Number of probes in probe-set) 2,

[00135] This allows including probe-sets in which the average of probe-sets in a group is greater than the average expression of background probes of similar GC content as the probe-set probes as the center of background for the probe-set and enables one to derive the probe-set dispersion from the background probe-set variance.

[00136] In some embodiments of the present disclosure, probe-sets that exhibit no, or low variance may be excluded from further analysis. Low-variance probe-sets are excluded from the analysis via a Chi-Square test. A probe-set is considered to be low-variance if its transformed variance is to the left of the 99 percent confidence interval of the Chi-Squared distribution with (N-l) degrees of freedom. (N- l)*Probe-set Variance/(Gene Probe-set Variance). about.Chi-Sq(N-l) where N is the number of input CEL files, (N-l) is the degrees of freedom for the Chi-Squared distribution, and the "probe-set variance for the gene" is the average of probe-set variances across the gene. In some embodiments of the present invention, probe-sets for a given gene or transcript cluster may be excluded from further analysis if they contain less than a minimum number of probes that pass through the previously described filter steps for GC content, reliability, variance and the like. For example in some embodiments, probe-sets for a given gene or transcript cluster may be excluded from further analysis if they contain less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or less than about 20 probes.

[00137] Methods of data analysis of gene expression levels or of alternative splicing may further include the use of a feature selection method and/or process as provided herein.

In some embodiments of the present invention, feature selection is provided by use of the LIMMA software package (Smyth, G. K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420).

[00138] Methods of data analysis of gene expression levels and or of alternative splicing may further include the use of a pre -classifier method and/or process (e.g., implemented by a pre-classifier analysis module). For example, a method and/or process may use a cell-specific molecular fingerprint to pre-classify the samples according to their composition and then apply a correction/normalization factor. This data/information may then be fed in to a final classification method and/or process which would incorporate that information to aid in the final diagnosis.

[00139] In certain embodiments, the methods of the present invention include the use of a pre-classifier method and/or process (e.g., implemented by a pre-classifier analysis module) that uses a molecular fingerprint to pre-classify the samples as smoker or non-smoker prior to application of a UIP / non-UIP classifier of the present invention.

[00140] Methods of data analysis of gene expression levels and/or of alternative splicing may further include the use of a classifier method and/or process (e.g., implemented by a classifier analysis module) as provided herein. In some embodiments of the present invention a diagonal linear discriminant analysis, k-nearest neighbor classifier, support vector machine (SVM) classifier, linear support vector machine, random forest classifier, or a probabilistic model-based method or a combination thereof is provided for classification of microarray data. In some embodiments, identified markers that distinguish samples (e.g. first ILD from second ILD, normal vs. ILD) or distinguish subtypes (e.g. IPF vs. NSIP) are selected based on statistical significance of the difference in expression levels between classes of interest. In some cases, the statistical significance is adjusted by applying a Benjamin Hochberg or another correction for false discovery rate (FDR).

[00141] In some cases, the classifier may be supplemented with a meta-analysis approach such as that described by Fishel and Kaufman et al. 2007 Bioinformatics 23(13): 1599-606. In some cases, the classifier may be supplemented with a meta-analysis approach such as a repeatability analysis. In some cases, the repeatability analysis selects markers that appear in at least one predictive expression product marker set.

[00142] Methods for deriving and applying posterior probabilities to the analysis of microarray data are known in the art and have been described for example in Smyth, G. K. 2004 Stat. Appi. Genet. Mol. Biol. 3: Article 3. In some cases, the posterior probabilities may be used to rank the markers provided by the classifier. In some cases, markers may be ranked according to their posterior probabilities and those that pass a chosen threshold may be chosen as markers whose differential expression is indicative of or diagnostic for samples that are for example IPF or NSIP. Illustrative threshold values include prior probabilities of 0.7, 0.75, 0.8, 0.85, 0.9, 0.925, 0.95, 0.975, 0.98, 0.985, 0.99, 0.995 or higher.

[00143] A statistical evaluation of the results of the molecular profiling may provide, but is not limited to providing, a quantitative value or values indicative of one or more of the following: the likelihood of diagnostic accuracy; the likelihood of an ILD; the likelihood of a particular ILD; the likelihood of the success of a particular therapeutic intervention, the likelihood the subject is a smoker, and the likelihood the subject is a non-smoker. Thus a physician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. Rather, the data is presented directly to the physician in its most useful form to guide patient care. The results of the molecular profiling can be statistically evaluated using a number of methods known to the art including, but not limited to: the students T test, the two sided T test, pearson rank sum analysis, hidden markov model analysis, analysis of q-q plots, principal component analysis, one way ANOVA, two way ANOVA, LIMMA and the like. [00182] In some embodiments of the present invention, the use of molecular profiling alone or in combination with cytological analysis may provide a classification, identification, or diagnosis that is between about 85% accurate and about 99% or about 100% accurate. In some cases, the molecular profiling process and/or cytology provide a classification, identification, diagnosis of an ILD that is about, or at least about 85%, 86%, 87%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.75%, 99.8%, 99.85%, or 99.9% accurate. In some embodiments, the molecular profiling process and/or cytology provide a classification, identification, or diagnosis of the presence of a particular ILD type (e.g. IPF; NSIP; HP) that is about, or at least about 85%, 86%, 87%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.75%, 99.8%, 99.85%, or 99.9% accurate.

[00144] In some cases, accuracy may be determined by tracking the subject over time to determine the accuracy of the original diagnosis. In other cases, accuracy may be established in a deterministic manner or using statistical methods. For example, receiver

operator characteristic (ROC) analysis may be used to determine the optimal assay parameters to achieve a specific level of accuracy, specificity, positive predictive value, negative predictive value, and/or false discovery rate.

[00145] In some embodiments of the present disclosure, gene expression products and compositions of nucleotides encoding for such products which are determined to exhibit the greatest difference in expression level or the greatest difference in alternative splicing between a first ILD and a second ILD (e.g., between IPF and NSIP), between ILD and normal, and/or between smoker and non-smoker may be chosen for use as molecular profiling reagents of the present disclosure. Such gene expression products may be particularly useful by providing a wider dynamic range, greater signal to noise, improved diagnostic power, lower likelihood of false positives or false negative, or a greater statistical confidence level than other methods known or used in the art.

[00146] In other embodiments of the present invention, the use of molecular profiling alone or in combination with cytological analysis may reduce the number of samples scored as non-diagnostic by about, or at least about 100%, 99%, 95%, 90%, 80%, 75%, 70%, 65%, or about 60% when compared to the use of standard cytological techniques known to the art. In some cases, the methods of the present invention may reduce the number of samples scored as intermediate or suspicious by about, or at least about 100%, 99%, 98%, 97%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, or about 60%, when compared to the standard cytological methods used in the art.

[00147] In some cases the results of the molecular profiling assays, are entered into a database for access by representatives or agents of a molecular profiling business, the individual, a medical provider, or insurance provider. In some cases assay results include sample classification, identification, or diagnosis by a representative, agent or consultant of the business, such as a medical professional. In other cases, a computer analysis of the data is provided automatically. In some cases the molecular profiling business may bill the individual, insurance provider, medical provider, researcher, or government entity for one or more of the following: molecular profiling assays performed, consulting services, data analysis, reporting of results, or database access.

[00148] In some embodiments of the present invention, the results of the molecular profiling are presented as a report on a computer screen or as a paper record. In some cases, the report may include, but is not limited to, such information as one or more of the

following: the number of genes differentially expressed, the suitability of the original sample, the number of genes showing differential alternative splicing, a diagnosis, a statistical confidence for the diagnosis, the likelihood the subject is a smoker, the likelihood of an ILD, and indicated therapies.

(iv) Categorization of Samples Based on Molecular Profiling Results

[00149] The results of the molecular profiling may be classified into one of the following: smoker, non-smoker, ILD, a particular type of ILD, a non-ILD, or non-diagnostic (providing inadequate information concerning the presence or absence of an ILD). In some cases, the results of the molecular profiling may be classified into IPF versus NSIP categories. In particular cases, the results may be classified as UIP or non-UIP.

[00150] In some embodiments of the present invention, results are classified using a trained classifier. Trained classifiers of the present invention implement methods and/or processes that have been developed using a reference set of known ILD and normal samples, known smoker and non-smoker samples, or combinations of known ILD and normal samples from smokers and/or non-smokers including, but not limited to, samples with one or more histopathologies. In some embodiments, training (e.g., using a classifier training module) comprises comparison of gene expression product levels in a first set biomarkers from a first ILD to gene expression product levels in a second set of biomarkers from a second ILD, where the first set of biomarkers includes at least one biomarker that is not in the second set. In some embodiments, training (e.g., using a classifier training module) comprises comparison of gene expression product levels in a first set biomarkers from a first ILD that is non-UIP to gene expression product levels in a second set of biomarkers from a second ILD that is UIP, where the first set of biomarkers includes at least one biomarker that is not in the second set. In some embodiments, training (e.g., using a classifier training module) further comprises comparison of gene expression product levels in a first set biomarkers from a first subject that is a smoker to gene expression product levels in a second set of biomarkers from a second subject that is a non-smoker, where the first set of biomarkers includes at least one biomarker that is not in the second set. In some embodiments, either the entire classifier or portions of the classifier can be trained (e.g., using a classifier training module) using comparisons of expression levels of biomarker panels within a classification panel against all other biomarker panels (or all other biomarker signatures) used in the classifier.

[00151] Classifiers suitable for categorization of samples include but are not limited to k-nearest neighbor classifiers, support vector machines, linear discriminant analysis, diagonal linear discriminant analysis, updown, naive Bayesian classifiers, neural network classifiers, hidden Markov model classifiers, genetic classifiers, or any combination thereof.

[00152] In some cases, trained classifiers of the present invention may incorporate data other than gene expression or alternative splicing data such as but not limited to DNA polymorphism data, sequencing data, scoring or diagnosis by cytologists or pathologists of the present invention, information provided by the pre -classifier method and/or process of the present disclosure, or information about the medical history of the subject of the present disclosure.

[00153] When classifying a biological sample for diagnosis of ILD, there are typically two possible outcomes from a binary classifier. Similarly, when classifying a biological sample for diagnosis of smoker, there are typically two possible outcomes from a binary classifier. When a binary classifier is compared with actual true values (e.g., values from a biological sample), there are typically four possible outcomes. If the outcome from a prediction is p (where "p" is a positive classifier output, such as a particular ILD) and the actual value is also p, then it is called a true positive (TP); however if the actual value is n then it is said to be a false positive (FP). Conversely, a true negative has occurred when both the prediction outcome and the actual value are n (where "n" is a negative classifier output, such as no ILD, or absence of a particular disease tissue as described herein), and false negative is when the prediction outcome is n while the actual value is p. In one embodiment, consider a diagnostic test that seeks to determine whether a person has a certain disease. A false positive in this case occurs when the person tests positive, but actually does not have the disease. A false negative, on the other hand, occurs when the person tests negative, suggesting they are healthy, when they actually do have the disease. In some embodiments, a Receiver Operator Characteristic (ROC) curve assuming real-world prevalence of subtypes can be generated by re-sampling errors achieved on available samples in relevant proportions.

[00154] The positive predictive value (PPV), or precision rate, or post-test probability of disease, is the proportion of patients with positive test results who are correctly diagnosed. It is the most important measure of a diagnostic method as it reflects the probability that a positive test reflects the underlying condition being tested for. Its value does however depend on the prevalence of the disease, which may vary. In one example, FP (false positive); TN

(true negative); TP (true positive); FN (false negative). False positive rate (α)=FP/(FP+TN)-specificity; False negative rate (β)=FN/(TP+FN)- sensitivity; Power= sensitivity = 1- β; Likelihood-ratio positive=sensitivity/(1-specificity); Likelihood-ratio negative=(1 -sensitivity)/specificity.

[00155] The negative predictive value is the proportion of patients with negative test results who are correctly diagnosed. PPV and NPV measurements can be derived using appropriate disease subtype prevalence estimates. An estimate of the pooled disease prevalence can be calculated from the pool of indeterminates which roughly classify into B vs M by surgery. For subtype specific estimates, in some embodiments, disease prevalence may sometimes be incalculable because there are not any available samples. In these cases, the subtype disease prevalence can be substituted by the pooled disease prevalence estimate.

[00156] In some embodiments, the level of expression products or alternative exon usage is indicative of one or the following: IPF, NSIP, or HP.

[00157] In some embodiments, the level of expression products or alternative exon usage is indicative that the subject is a smoker or a non-smoker.

[00158] In some embodiments, the results of the expression analysis of the subject methods provide a statistical confidence level that a given diagnosis is correct. In some embodiments, such statistical confidence level is at least about, or more than about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% 99.5%, or more.

Reports

[00159] A subject method and/or system may include generating a report that provides an indication that a sample (a lung tissue sample) is an ILD sample (e.g., using a report module). A subject diagnostic method can include generating a report that provides an indication as to whether an individual being tested has an ILD. A subject diagnostic method can include generating a report that provides an indication as to whether an individual being tested is, or is not a smoker. A subject method (or report module) can include generating a report that provides an indication as to whether an individual being tested has IPF (and not, e.g., an ILD other than IPF; e.g., the report can indicate that the individual has IPF and not NSIP).

[00160] In some embodiments, a subject method of diagnosing an ILD involves generating a report (e.g., using a report module). Such a report can include information such as a likelihood that the patient has an ILD; a likelihood that the patient is a smoker; a recommendation regarding further evaluation; a recommendation regarding therapeutic drug and/or device intervention; and the like.

[00161] For example, the methods disclosed herein can further include a step of generating or outputting a report providing the results of a subject diagnostic method, which report can be provided in the form of an electronic medium (e.g., an electronic display on a computer monitor), or in the form of a tangible medium (e.g., a report printed on paper or other tangible medium). An assessment as to the results of a subject diagnostic method (e.g., a likelihood that an individual has an ILD; a likelihood that an individual has IPF; a likelihood that an individual is a smoker) can be referred to as a "report" or, simply, a "score." A person or entity that prepares a report ("report generator") may also perform steps such as sample gathering, sample processing, and the like. Alternatively, an entity other than the report generator can perform steps such as sample gathering, sample processing, and the like. A diagnostic assessment report can be provided to a user. A "user" can be a health professional (e.g., a clinician, a laboratory technician, a physician (e.g., a cardiologist), etc.).

[00162] A subject report can further include one or more of: 1) service provider information; 2) patient data; 3) data regarding the expression level of a given gene product or set of gene products, a score or classifier decision; 4) follow-up evaluation recommendations; 5) therapeutic intervention or recommendations; and 6) other features.

Further Evaluation

[00163] Based on the expression level of a given gene product or set of gene products, and/or based on a report (as described above), a physician or other qualified medical personnel can determine whether further evaluation of the test subject (the patient) is required. Further evaluation can include, e.g., spirometry.

Therapeutic intervention

[00164] Based on the expression level of a given gene product or set of gene products, and/or based on a report (as described above), a physician or other qualified medical personnel can determine whether appropriate therapeutic intervention is advised.

[00165] Therapeutic intervention includes drug-based therapeutic intervention, device-based therapeutic intervention, and surgical intervention. Where a report indicates a likelihood that an individual has IPF, drug-based therapeutic intervention includes, e.g., administering to the individual an effective amount of pirfenidone, prednisone, azathioprine, or N-acetylcysteine. Surgical intervention includes, e.g., arterial bypass surgery.

Computer-Implemented Methods, Systems and Devices

Therapeutic intervention

[00166] The methods of the present disclosure can be computer-implemented, such that method steps (e.g., assaying, comparing, calculating, and the like) are be automated in whole or in part.

[00167] Accordingly, the present disclosure provides methods, computer systems, devices and the like in connection with computer-implemented methods of facilitating a diagnosis of an interstitial lung disease (e.g., a diagnosis of IPF, NSIP, HP, etc.), including differential diagnosis.

[00168] The present disclosure further provides methods, computer systems, devices and the like in connection with computer-implemented methods of facilitating determination of smoker status (e.g., smoker vs. non-smoker).

[00169] The present disclosure further provides methods, computer systems, devices and the like in connection with computer-implemented methods of facilitating a diagnosis of an interstitial lung disease (e.g., a diagnosis of IPF, NSIP, HP, etc.), including differential diagnosis, wherein the methods further comprise determining a subjects smoker status (smoker vs. non-smoker) and incorporating smoker status into the determination of the subjects interstitial lung disease diagnosis. In some embodiments, (i) smoker status is incorporated into the interstitial lung disease diagnosis as a covariate in the model used during training (e.g., using a classifier training module). This approach boosts signal-to-noise ratio, particularly in data derived from smokers (were noise is higher) and allows data derived from smokers and non-smokers to be combined and used simultaneously. In some embodiments, (ii) smoker status is incorporated into the interstitial lung disease diagnosis by identifying one or more genes that are susceptible to smoker status bias and excluding such genes or weighing such genes differently than other genes that are not susceptible to smoker-status during interstitial lung disease diagnosis classifier training. In some embodiments, (iii)

smoker status is incorporated into the interstitial lung disease diagnosis by constructing a tiered classification in which an initial classifier is trained to recognize the gene signatures that distinguish smokers from non-smokers (e.g., using a classifier training module). Once patient samples are pre-classified as "smoker" or "non-smoker" (e.g., using a pre-classifier analysis module), distinct classifiers that were each trained to distinguish UIP vs. Non UIP in smokers or non-smokers, respectively can be implemented to diagnose interstitial lung disease. In still further embodiments, such methods comprising the step of incorporating smoker status into the determination of the subjects interstitial lung disease diagnosis include a combination of one or more of the above mentioned means of such incorporation (i.e., a combination of two or more of embodiments (i) to (iii) in the instant paragraph.

[0100] For example, the method steps, including obtaining values for biomarker levels, comparing normalized biomarker (gene) expression levels to a control level, calculating the likelihood of an ILD (and optionally the likelihood a subject is a smoker), generating a report, and the like, can be completely or partially performed by a computer program product. Values obtained can be stored electronically, e.g., in a database, and can be subjected to a classifier executed by a programmed computer (e.g., using a classifier analysis module).

[0101] For example, the methods and/or systems of the present disclosure can involve inputting a biomarker level (e.g., a normalized expression level of a gene product) into a classifier analysis module to execute a method and/or process to perform the comparing and calculating step(s) described herein, and generate a report (e.g., using a report module) as described herein, e.g., by displaying or printing a report to an output device at a location local or remote to the computer. The output to the report can be a score (e.g., numerical score (representative of a numerical value) or a non-numerical score (e.g., non-numerical output (e.g., "IPF", "No evidence of IPF") representative of a numerical value or range of numerical values. In other aspects, the output may indicate "UIP" vs. "non-UIP." In other aspects, the output may indicate "Smoker" vs. "Non-smoker"

[0102] The present disclosure thus provides a computer program product including a computer readable storage medium having software and/or hardware modules stored on it. The software and/or hardware modules can, when executed by a processor, execute relevant calculations based on values obtained from analysis of one or more biological sample (e.g., lung tissue sample) from an individual. The computer program product has stored therein a computer program for performing the calculation(s).

[0103] The present disclosure provides systems for executing the program described above, which system generally includes: a) a central computing environment or processor executing software and/or hardware modules; b) an input device, operatively connected to the computing environment, to receive patient data, wherein the patient data can include, for example, biomarker level or other value obtained from an assay using a biological sample from the patient, as described above; c) an output device, connected to the computing environment, to provide information to a user (e.g., medical personnel); and d) a method and/or process executed by the central computing environment (e.g., a processor), where the method and/or process is executed based on the data received by the input device, and wherein the method and/or process calculates a value, which value is indicative of the likelihood the subject has an ILD, as described herein.

[0104] The present disclosure also provides systems for executing the program described above, which system generally includes: a) a central computing environment or processor executing software and/or hardware modules; b) an input device, operatively connected to the computing environment, to receive patient data, wherein the patient data can include, for example, biomarker level or other value obtained from an assay using a biological sample from the patient, as described above; c) an output device, connected to the computing environment, to provide information to a user (e.g., medical personnel); and d) a method and/or process executed by the central computing environment (e.g., a processor), where the method and/or process is executed based on the data received by the input device, wherein the method and/or process calculates a value, which value is indicative of the likelihood the subject has an ILD, as described herein, and wherein the method and/or process uses smoking status (smoker vs. non-smoker) as a covariate in the model used during training. In some embodiments, the method and/or process excludes or weighs one or more gene that is susceptible to smoker status bias differently during classifier training to enrich the feature space used for training with genes that are not confounded or affected by smoking status.

[0105] In still further embodiments, the present disclosure provides systems for executing the program described above, which system generally includes: a) a central computing environment or processor executing software and/or hardware modules; b) an input device, operatively connected to the computing environment, to receive patient data, wherein the patient data can include, for example, biomarker level or other value obtained from an assay using a biological sample from the patient, as described above; c) an output device, connected to the computing environment, to provide information to a user (e.g., medical personnel); and d) a first method and/or process executed by the central computing environment (e.g., a

processor), where the first method and/or process is executed based on the data received by the input device, wherein the first method and/or process calculates a value, which value is indicative of the likelihood a subject is a smoker or a non-smoker, as described herein, wherein the subject's status as a smoker or non-smoker causes the first method and/or process to apply a second method and/or process specifically trained (e.g., using a classifier training module) to distinguish UIP vs. Non UIP in smokers or non-smokers, respectively and e) wherein the second method and/or process is executed by the central computing environment (e.g., a processor), where the second method and/or process is executed based on the data received by the input device, and wherein the second method and/or process calculates a value, which value is indicative of the likelihood the subject has an ILD, as described herein,

Computer Systems

[0106] Figure 7A illustrates a processing system 100 including at least one processor 102, or processing unit or plurality of processors, memory 104, at least one input device 106 and at least one output device 108, coupled together via a bus or group of buses 110. Processing system can be implemented on any suitable device, such as, for example, a host device, a personal computer, a handheld or laptop device, a personal digital assistant, a multiprocessor system, a microprocessor-based system, a programmable consumer electronic device, a minicomputer, a server computer, a web server computer, a mainframe computer, and/or a distributed computing environment that includes any of the above systems or devices

[0107] In certain embodiments, input device 106 and output device 108 can be the same device. An interface 112 can also be provided for coupling the processing system 100 to one or more peripheral devices, for example interface 112 can be a PCI card or PC card. At least one storage device 114 which houses at least one database 116 can also be provided.

[0108] The memory 104 can be any form of memory device, for example, volatile or nonvolatile memory, solid state storage devices, magnetic devices, etc. For example, in some embodiments, the memory 104 can be a random access memory (RAM), a memory buffer, a hard drive, a read-only memory (ROM), an erasable programmable read-only memory (EPROM), a database, and/or the like.

[0109] The processor 102 can include more than one distinct processing device, for example to handle different functions within the processing system 100. The processor 100 can be any suitable processing device configured to run or execute a set of instructions or code (e.g., stored in the memory) such as a general-purpose processor (GPP), a central processing unit (CPU), an accelerated processing unit (APU), a graphics processor unit (GPU), an Application Specific Integrated Circuit (ASIC), and/or the like. Such a processor 100 can run

or execute a set of instructions or code stored in the memory associated with using a personal computer application, a mobile application, an internet web browser, a cellular and/or wireless communication (via a network), and/or the like. More specifically, the processor can execute a set of instructions or code stored in the memory 104 associated with analyzing and classifying data, as described herein.

[0110] Input device 106 receives input data 118 and can comprise, for example, a keyboard, a pointer device such as a pen-like device or a mouse, audio receiving device for voice controlled activation such as a microphone, data receiver or antenna such as a modem or wireless data adaptor, data acquisition card, etc. Input data 118 can come from different sources, for example keyboard instructions in conjunction with data received via a network.

[0111] Output device 108 produces or generates output data 120 and can comprise, for example, a display device or monitor in which case output data 120 is visual, a printer in which case output data 120 is printed, a port for example a USB port, a peripheral component adaptor, a data transmitter or antenna such as a modem or wireless network adaptor, etc. Output data 120 can be distinct and derived from different output devices, for example a visual display on a monitor in conjunction with data transmitted to a network. A user can view data output, or an interpretation of the data output, on, for example, a monitor or using a printer.

[0112] In some embodiments, the input device 106 and/or the output device 108 can be a communication interface configured to send and/or receive data via a network. More specifically, in such embodiments, the processing system 100 can act as a host device to one or more client devices (not shown in Figure 7A). As such, the processing system 100 can send data to (e.g., output data 120) and receive data from (e.g., input data 118) the client devices. Such a communication interface can be any suitable module and/or device that can place the processing system 100 in communication with a client device such as one or more network interface cards or the like. Such a network interface card can include, for example, an Ethernet port, a WiFi® radio, a Bluetooth® radio, a near field communication (NFC) radio, and/or a cellular radio that can place the client device 150 in communication with the host device 110 via a network or the like.

[0113] The storage device 114 can be any form of data or information storage means, for example, volatile or non-volatile memory, solid state storage devices, magnetic devices, etc. For example, in some embodiments, the storage device 114 can be a random access memory (RAM), a memory buffer, a hard drive, a read-only memory (ROM), an erasable programmable read-only memory (EPROM), a database, and/or the like.

[0114] In use, the processing system 100 is adapted to allow data or information to be stored in and/or retrieved from, via wired or wireless communication means, at least one database 116. The interface 112 may allow wired and/or wireless communication between the processing unit 102 and peripheral components that may serve a specialized purpose. In general, the processor 102 can receive instructions as input data 118 via input device 106 and can display processed results or other output to a user by utilizing output device 108. More than one input device 106 and/or output device 108 can be provided. The processing system 100 may be any suitable form of terminal, server, specialized hardware, or the like. The processing system 100 may be a part of a networked communications system.

[0115] Processing system 100 can connect to a network, for example, a local area network (LAN), a virtual network such as a virtual local area network (VLAN), a wide area network (WAN), a metropolitan area network (MAN), a worldwide interoperability for microwave access network (WiMAX), a cellular network, the Internet, and/or any other suitable network implemented as a wired and/or wireless network. For instance, when used in a LAN networking environment, the computing system environment 100 is connected to the LAN through a network interface or adapter. When used in a WAN networking environment, the computing system environment typically includes a modem or other means for establishing communications over the WAN, such as the Internet. The modem, which may be internal or external, may be connected to a system bus via a user input interface, or via another appropriate mechanism. In a networked environment, program modules depicted relative to the computing system environment 100, or portions thereof, may be stored in a remote memory storage device. It is to be appreciated that the illustrated network connections of Fig. 7 are examples and other means of establishing a communications link between multiple computers may be used.

[0116] Input data 118 and output data 120 can be communicated to other devices via the network. The transfer of information and/or data over the network can be achieved using wired communications means or wireless communications means. A server can facilitate the transfer of data between the network and one or more databases. A server and one or more databases provide an example of an information source.

[0117] Thus, the processing computing system environment 100 illustrated in Fig. 7A may operate in a networked environment using logical connections to one or more remote computers. The remote computer may be a personal computer, a server, a router, a network PC, a peer device, or other common network node, and typically includes many or all of the elements described above.

[0118] FIG. 7B illustrates the processor 102 of FIG. 7A in greater detail. The processor 102 can be configured to execute specific modules. The modules can be, for example, hardware modules, software modules stored in the memory 104 and/or executed in the processor 102, and/or any combination thereof. For example, as shown in FIG. 7B, the processor 102 includes and/or executes a pre-classifier analysis module 130, a classifier training module 132, a classifier analysis module 134 and a report module 136. As shown in FIG. 7B, the pre-classifier analysis module 130, the classifier training module 132, the classifier analysis module 134 and the report module 136 can be connected and/or electrically coupled. As such, signals can be sent between the pre-classifier analysis module 130, the classifier training module 132, the classifier analysis module 134 and the report module 136.

[0119] The classifier training module 132 can be configured to receive a corpora of data (e.g. gene expression data, sequencing data) and train a classifier. For example, clinical annotation data from samples previously identified as UIP and non-UIP (e.g., by an expert) can be received by the input device 106 and used by the classifier training module 132 to identify correlations between the samples previously identified as UIP and non-UIP. For example, expert TBB histopathology labels (i.e., UIP or Non UIP), expert HRCT labels, and/or expert patient-level clinical outcome labels can be obtained and used alone or in combination to train the classifier using microarray and/or sequencing data. The feature space used can include gene expression, variants, mutations, fusions, loss of heterozygoxity (LOH), biological pathway effect and/or any other dimension of the data that can be extracted as a feature for the purposes of training a machine-learning algorithm. In some embodiments, the feature space used for training a UIP vs. Non-UIP classifier, a smoker vs. Non-smoker classifier, or a UIP vs. Non-UIP and smoker vs. Non-smoker classifier includes gene expression, variants, mutations, fusions, loss of heterozygoxity (LOH), and biological pathway effect. In some embodiments, the feature space used for training a UIP vs. Non-UIP classifier, a smoker vs. Non-smoker classifier, or a UIP vs. Non-UIP and smoker vs. Non-smoker classifier includes gene expression and variant dimensions.

[0120] In some embodiments, the classifier training module 132 can train a smoker classifier and a non-smoker classifier based on an indication associated with whether a received sample is associated with a smoker or non-smoker. In other embodiments, the smoker/non-smoker can be used as an attribute (a model covariate) to train a single classifier. After the classifier is trained, it can be used to identify and/or classify newly received and unknown samples as described herein.

[0121] The pre-classifier analysis module 130 can identify whether a sample is associated with a smoker or a non-smoker. Specifically, the pre-classifier analysis module 130 can use any suitable method to identify and/or classify a sample as coming from an individual that smokes (or has a past history of heavy smoking) versus an individual that does not smoke (or has no smoking history). The classification can be done in any suitable manner such as, receiving an indication from a user, identification of genes that are susceptible to smoker-status bias, using a machine-learning classifier, and/or any other suitable method described herein.

[0122] The classifier analysis module 134 can input the sample into the classifier to identify and/or classify the received sample as associated with UIP and non-UIP. Specifically, the classifier analysis module 134 can use a trained classifier to identify whether the sample indicates UIP or non-UIP. In some embodiments, the classifier analysis module 134 can indicate a percentage or confidence score of the sample being associated with UIP or non-UIP. In some embodiments, the classifier analysis module 134 can execute two separate classifiers: one for smoker samples and the other for non-smoker samples (as determined by the pre-classifier analysis module 130). In other embodiments, a single classifier is executed for both smoker and non-smoker samples with an input for smoker status.

[0123] The report module 136 can be configured to generate any suitable report based on the outcome of the classifier analysis module 134 as described in further detail herein. In some cases, the report may include, but is not limited to, such information as one or more of the following: the number of genes differentially expressed, the suitability of the original sample, the number of genes showing differential alternative splicing, a diagnosis, a statistical confidence for the diagnosis, the likelihood the subject is a smoker, the likelihood of an ILD, and indicated therapies.

[0124] FIG. 7C illustrates a flow chart of one non-limiting embodiment of the present invention wherein gene product expression data for known UIP and non-UIP samples are used to train (e.g., using a classifier training module) a classifier for differentiating UIP vs. non-UIP, wherein the classifier optionally considers smoker status as a covariant, and wherein gene product expression data from unknown samples are input into the trained classifier to identify the unknown samples as either UIP or non-UIP, and wherein the results of the classification via the classifier are defined and output via a report.

[0125] Certain embodiments may be described with reference to acts and symbolic representations of operations that are performed by one or more computing devices, such as the computing system environment 100 of Fig. 7A. As such, it will be understood that such

acts and operations, which are at times referred to as being computer-executed, include the manipulation by the processor of the computer of electrical signals representing data in a structured form. This manipulation transforms the data or maintains them at locations in the memory system of the computer, which reconfigures or otherwise alters the operation of the computer in a manner understood by those skilled in the art. The data structures in which data is maintained are physical locations of the memory that have particular properties defined by the format of the data. However, while an embodiment is being described in the foregoing context, it is not meant to be limiting as those of skill in the art will appreciate that the acts and operations described hereinafter may also be implemented in hardware.

[0126] Embodiments may be implemented with numerous other general-purpose or special-purpose computing devices and computing system environments or configurations. Examples of other computing systems, environments, and configurations that may be suitable for use with an embodiment include, but are not limited to, personal computers, handheld or laptop devices, personal digital assistants, multiprocessor systems, microprocessor-based systems, programmable consumer electronics, network, minicomputers, server computers, web server computers, mainframe computers, and distributed computing environments that include any of the above systems or devices.

[0127] Embodiments may be described in a general context of computer-executable instructions, such as hardware and/or software modules. An embodiment may also be practiced in a distributed computing environment where tasks are performed by remote processing devices that are linked through a communications network. In a distributed computing environment, program modules may be located in both local and remote computer storage media including memory storage devices.

Computer program products

[0128] The present disclosure provides computer program products that, when executed on a programmable computer such as that described above with reference to Fig. 7, can carry out the methods of the present disclosure. As discussed above, the subject matter described herein may be embodied in systems, apparatus, methods, and/or articles depending on the desired configuration. These various implementations may include implementation in one or more computer programs that are executable and/or interpretable on a programmable system including at least one programmable processor, which may be special or general purpose, coupled to receive data and instructions from, and to transmit data and instructions to, a

storage system, at least one input device (e.g. video camera, microphone, joystick, keyboard, and/or mouse), and at least one output device (e.g. display monitor, printer, etc.).

[0129] Computer programs (also known as programs, software, software applications, applications, components, or code) include instructions for a programmable processor, and may be implemented in a high-level procedural and/or object-oriented programming language, and/or in assembly/machine language. As used herein, the term "machine -readable medium" refers to any computer program product, apparatus and/or device (e.g., magnetic discs, optical disks, memory, etc.) used to provide machine instructions and/or data to a programmable processor, including a machine-readable medium that receives machine instructions as a machine -readable signal.

[0130] It will be apparent from this description that aspects of the present disclosure may be embodied, at least in part, in software, hardware, firmware, or any combination thereof. Thus, the techniques described herein are not limited to any specific combination of hardware circuitry and/or software, or to any particular source for the instructions executed by a computer or other data processing system. Rather, these techniques may be carried out in a computer system or other data processing system in response to one or more processors, such as a microprocessor, executing sequences of instructions stored in memory or other computer-readable medium including any type of ROM, RAM, cache memory, network memory, floppy disks, hard drive disk (HDD), solid-state devices (SSD), optical disk, CD-ROM, and magnetic -optical disk, EPROMs, EEPROMs, flash memory, or any other type of media suitable for storing instructions in electronic format.

[0131] In addition, the processor(s) may be, or may include, one or more programmable general-purpose or special-purpose microprocessors, digital signal processors (DSPs), programmable controllers, application specific integrated circuits (ASICs), programmable logic devices (PLDs), trusted platform modules (TPMs), or the like, or a combination of such devices. In alternative embodiments, special- purpose hardware such as logic circuits or other hardwired circuitry may be used in combination with software instructions to implement the techniques described herein.

Arrays and Kits

[0132] The present disclosure provides arrays and kits for use in carrying out a subject evaluating method or a subject diagnostic method.

Arrays

[0133] A subject array can comprise a plurality of nucleic acids, each of which hybridizes to a gene differentially expressed in a cell present in a tissue sample obtained from an individual being tested for an ILD.

[0134] A subject array can comprise a plurality of nucleic acids, each of which hybridizes to a gene differentially expressed in a cell present in a tissue sample obtained from an individual being tested for smoker status.

[0135] A subject array can comprise a plurality of nucleic acids, each of which hybridizes to a gene differentially expressed in a cell present in a tissue sample obtained from an individual being tested for both smoker status and an ILD.

[0136] A subject array can comprise a plurality of member nucleic acids, each of which member nucleic acids hybridizes to a different gene product. In some cases, two or more member nucleic acids hybridize to the same gene product; e.g., in some cases 2, 3, 4, 5, 6, 7, 8, 9, 10, or more member nucleic acids hybridize to the same gene product. A member nucleic acid can have a length of from about 5 nucleotides (nt) to about 100 nt, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 18, 19, 20, 20-25, 25-30, 30-40, 40-50, 50-60, 60-70, 70- 80, 80-90, or 90-100 nt. A nucleic acid can have one or more phosphate backbone modifications.

[0137] A subject array can include from about 10 to about 10^5 unique member nucleic acids, or more than 10^5 unique member nucleic acids. For example, a subject array can include from about 10 to about 10^2, from about 10^2 to about 10^3, from about 10^3 to about 10^4, from about 10^4 to about 10^5, or more than 10^5, unique member nucleic acids.

Abbreviations

adj.P.Value.edgeR:	False discovery rate adjusted p value of RNAseq gene expression data using edgeR analysis.
adj.P.Value.microarray	False discovery rate adjusted p value of RNAseq gene expression data using microarray analysis
adj.P.Value.npSeq:	False discovery rate adjusted p value of RNAseq gene expression data using npSeq analysis
BRONCH:	Broncholitis
CIF-NOC	Chronic Interstitial Fibrosis Not Otherwise Classified
edgeR:	an R package for the significance analysis of sequencing data

Ensembl ID:	Gene Identifier from Ensembl Genome Browser database
FDR:	False Discovery Rate, an adjusted p value that limits the possibility that the results are random due to the large number of genes simultaneously evaluated.
Gene Symbol:	Gene Identifier from HUGO Gene Nomenclature Committee
logFC.edgeR:	Log2 fold change of RNAseq gene expression data using edgeR analysis
logFC.microarray:	Log2 fold change of RNAseq gene expression data using LIMMA microarray analysis
logFC.npSeq:	Log2 fold change of RNAseq gene expression data using npSeq analysis
microarray:	Gene expression analysis using gene arrays such as from Affymetrix.
NML:	Normal Lung, usually obtained from human lung donor tissue that was ultimately never transplanted
npSeq:	an R package for the significance analysis of sequencing data
NSIP:	Non Specific Interstitial Pneumonia
OP:	Organizing Pneumonia
P.value.edgeR:	p value of RNAseq gene expression data using edgeR analysis
P.value.microarray:	p value of RNAseq gene expression data using LIMMA microarray analysis
P.value.npSeqp:	value of RNAseq gene expression data using npSeq analysis
RB:	Respiratory Bronchiolitis
REST:	A combination of all other ILDs except the subtype it is being compared to. Usually HP and NSIP, BRONCH, CIF-NOC, OP, RB and SARC.
SARC:	Sarcoidosis
SQC:	Squamous Cell Carcinoma
TCID:	"TCID" or "Transcript Cluster Identifier" refers to a gene level identifier used by all Affymetrix microarrays. Each TCID is associated with a fixed reference number that identifies a set of specific probes having sequences for a specific gene. Such specific probes are present on a given array commercially available from Affymetrix. TCID numbers thus refer to a gene product(s) of a specific gene, and can be found, e.g., at the following world wide web address: affymetrix.com/ the sequences of which probes and gene products are hereby incorporation herein in their entirety.

UIP:	Usual Interstitial Pneumonia; the HRCT or histopathology pattern observed in IPF
LIMMA:	Linear Models for Microarray Data; an R package for the significance analysis of microarray data.

[0138] "ENSEMBL ID" refers to a gene identifier number from the Ensembl Genome Browser database (see world wide web address: ensembl.org/index.html, incorporate herein). Each identifier begins with the letters ENSG to denote "Ensembl Gene". Each ENSEMBL ID number (i.e., each "gene" in the Ensembl database) refers to a gene defined by a specific start and stop position on a particular human chromosome, and therefore defines a specific locus of the human genome. As one of average skill in the art would fully appreciate, all of the gene symbols disclosed herein refer to gene sequences, which are readily available on publically available databases, e.g., UniGene database (Pontius JU, Wagner L, Schuler GD. UniGene: a unified view of the transcriptome. In: The NCBI Handbook. Bethesda (MD): National Center for Biotechnology Information; 2003, available at the world wide web address ncbi.nlm.nih.gov/unigene, incorporated herein), RefSeq (The NCBI handbook [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project, available at the world wide web address: ncbi.nlm.nih.gov/refseq/, incorporate herein), Ensembl (EMBL, available at the world wide web address: ensembl.org/index.html, incorporated herein), and the like. The sequences of the genes disclosed herein via their gene symbols, Ensembl IDs, and Entrez IDs are herein incorporated in their entirety.

[0139] All references, patents, and patent applications cited herein are incorporated in their entirety for all purposes.

EXAMPLES

EXAMPLE 1

Sample Collection, Pathology Diagnosis, and Labeling

[0140] Video-assisted thoracoscopic surgery (VATS) specimens were prospectively collected as a part of an Institutional Review Board (IRB) approved ongoing multi-center clinical protocol, BRonchial sAmple collection for a noVel gEnomic test (BRAVE), sponsored by Veracyte, Inc. (South San Francisco, CA). Additional VATS and surgical lung biopsy specimens were obtained from banked sources.

[0141] Following surgery, histology slides were collected, de-identified, and submitted to expert pathology review. Selected slides were scanned to construct a permanent digital file of microscopic images (Aperio, Vista, CA). Slides were evaluated according to the central pathology diagnostic process described in Figure 5, resulting in both sample-level and patient-level pathology diagnoses. Pathology categories are summarized in Table 3. A patient can have more than one sample-level diagnosis (i.e. one per VATS sample per patient, most often one from each of the lower and upper lobes of the right lung), but can only have one patient-level diagnosis.

[0142] Table 3. List of all pathology diagnoses considered in our central pathology diagnostic process.

Diagnosis	Abbreviation
Classic Usual Interstitial Pneumonia	Classic UIP
Difficult Usual Interstitial Pneumonia	Difficult UIP
Favor Usual Interstitial Pneumonia	Favor UIP
Cellular Non-specific Interstitial Pneumonia	Cellular NSIP
Fibrotic Non-specific Interstitial Pneumonia	Fibrotic NSIP
Both cellular and fibrotic Non-specific Interstitial Pneumonia	Both cellular and fibrotic NSIP
Favor Non-specific Interstitial Pneumonia	Favor NSIP
Hypersensitivity Pneumonitis	HP
Favor Hypersensitivity Pneumonitis	Favor HP
Chronic Interstitial Fibrosis, Not Otherwise Classified	CIF/NOC
Organizing Pneumonia	OP
Diffuse Alveolar Damage	DAD
Respiratory Bronchiolitis	RB
Smoking-Related Interstitial Fibrosis	SRIF
Emphysema	Emphysema
Bronchiolitis	Bronchiolitis
Sarcoidosis	Sarcoidosis
Lymphangioleiomyomatosis	LAM
Langerhans cell histiocytosis	LCH
Eosinophilic Pneumonia	EP
Non-diagnostic	ND
Other	Other

[0143] Most diagnostic terminologies follow American Thoracic Society (ATS) 2011 or 2013 guidelines[5,6], but a few changes were made by the expert pathologist panel to better characterize features at the lobe level. In particular, 'Classic UIP' and 'Difficult UIP' were included instead of 'Definite UIP' and 'Probable UIP' as described in the ATS 2011

guidelines. Chronic Interstitial Fibrosis, Not Otherwise Classified (CIF/NOC) corresponds to unclassifiable fibrotic ILD. Three subcategories of CIF/NOC, 'Favor UIP', 'Favor NSIP', and 'Favor HP', were defined to specify cases of unclassifiable fibrosis which, in the judgment of the expert pathology panel, exhibit features suggestive of UIP, non-specific interstitial pneumonia (NSIP), or hypersensitivity pneumonitis (HP). A diagnosis of Smoking-Related Interstitial Fibrosis (SRIF) is also included[20].

[0144] For classification, sample-level pathology diagnoses were converted into binary class labels (UIP and non-UIP). Among the pathology diagnosis categories (Table 3), the 'UIP' class includes (1) UIP, (2) Classic UIP, (3) Difficult UIP, and (4) CIF/NOC, Favor UIP. All other pathology diagnoses except Non-diagnostic (ND) were assigned to the 'non-UIP' class.

EXAMPLE 2

Sample Processing

[0145] Frozen tissue samples were mounted for sectioning using Tissue-Tek O.C.T. medium (Sakura Finetek U.S.A.) and 2 x 20μm sections generated using a CM1800 cryostat (Leica Biosystems, Buffalo Grove, Illinois). Tissue curls were immediately immersed in RNAprotect (QIAGEN, Valencia, California), incubated overnight at 4°C and stored at -80°C until extraction. Whenever possible, adjacent 5μm tissue curls were mounted onto glass slides and processed for hematoxylin and eosin (H&E) staining following standard procedures.

[0146] Nucleic acids were extracted using the AllPrep Micro Kit (QIAGEN) according to manufacturer's guidelines. Total RNA yield and quality was determined using Quant-it (Invitrogen) and Pico BioAnalyzer kits (Agilent). Fifteen nanograms of total RNA were amplified using Ovation FFPE WTA System (NuGEN, San Carlos, California), hybridized to GeneChip Gene ST 1.0 (Affymetrix, Santa Clara, California) microarrays, processed and scanned according to the manufacturer's protocols. Expression data was normalized by Robust Multi-array Average (RMA).

EXAMPLE 3

Next-Generation RNA Sequencing

[0147] Whole transcriptome RNA sequencing was performed on select samples at a targeted minimum read depth of 80 million paired-end reads per sample. Briefly, 10ng of total RNA was amplified using the Ovation RNASeq System v2 (NuGEN, San Carlos, California) and TruSeq (Illumina, San Diego, California) sequencing libraries were prepared and sequenced on an Illumina HiSeq according to manufacturer's instructions. Raw reads were aligned to the hg19 genome assembly using TopHat2. Gene counts were obtained using HTSeq and normalized in Bioconductor using the *varianceStabilizingTransformation* function in the DESeq2 package. Raw counts and normalized expression levels were obtained for 55,097 transcripts.

EXAMPLE 4

Cohort Selection and Classifier Training

[0148] The study cohort initially included both banked (n=128) and prospectively collected BRAVE (n=38) tissues. Banked samples with poor cellularity on H&E staining (n=4 from a single patient) or normal lung tissue appearance (n=1) were excluded, as were samples diagnosed as 'unclassifiable fibrotic ILD' i.e. CIF/NOC (n=3) or samples that lacked pathology agreement by at least two pathologists (n=29). For BRAVE samples, CIF/NOC samples were not excluded. Only one BRAVE cohort sample was omitted, due to missing central pathology diagnosis. Processed RNA samples with residual genomic DNA contamination (n=2) or low RNA quality (RNA integrity number (RIN) < 4) (n=1) were also excluded. After all exclusions, 125 samples from 86 patients remained for use in classification. The age, gender, smoking history and pathology diagnoses of included patients are summarized in Table 1.

[0149] Table 1. Cohort summary. Within each set of microarray data or RNASeq data, clinical factors such as age, gender and smoking history are summarized across patients. In addition, samples are summarized by sample-level pathology diagnosis (counts without parenthesis), and patients are summarized by patient-level pathology diagnosis (counts within parenthesis). Zeros in either case are due to discordance between sample-level and patient-level pathology; counts will therefore not be additive. Of the 36 samples in the RNASeq training set, 22 overlap with the microarray training set and 14 overlap with the microarray test set.

	Microarray	RNASeq

Category	Sub-category	All	Training	Test	Training
Size	Patient	86	54	32	29
Age	Mean (range)	57.9 (25-83)	58.9 (25-83)	56.3 (32-76)	60.5 (32-80)
Gender	Male	32 (37.2%)	22 (40.7%)	10 (31.2%)	16 (55.2%)
	Female	54 (62.8%)	32 (59.3%)	22 (68.8%)	13 (44.8%)
Smoking	Yes	45 (52.3%)	33 (61.1%)	12 (37.5%)	15 (51.7%)
	No	38 (44.2%)	19 (35.2%)	19 (59.4%)	14 (48.3%)
	Unknown	3 (3.5%)	2 (3.7%)	1 (3.1%)	0
Pathology diagnosis	UIP	45 (30)	28 (18)	17 (12)	14 (14)
	Classic UIP	8 (5)	5 (4)	3 (1)	2 (0)
	Difficult UIP	5 (2)	3 (1)	2 (1)	1 (0)
	Favor UIP	3 (1)	3 (1)	0 (0)	0 (0)
	Fibrotic NSIP	2 (0)	0 (0)	2 (0)	0 (0)
	Cellular NSIP	7 (0)	5 (0)	2 (0)	2 (0)
	Both cellular and fibrotic NSIP	14 (0)	9 (0)	5 (0)	3 (0)
	NSIP	0 (15)	0 (10)	0 (5)	0 (5)
	Favor HP	2 (1)	0 (0)	2 (1)	0 (0)
	HP	16 (14)	11 (10)	5 (4)	3 (2)
	Unclassifiable fibrotic ILD	4 (5)	2 (3)	2 (2)	0 (0)
	Sarcoidosis	4 (4)	2 (2)	2 (2)	2 (2)
	RB	4 (2)	0 (1)	4 (1)	3 (1)
	OP	2 (1)	2 (1)	0 (0)	1 (1)
	SRIF	1 (1)	0 (0)	1 (1)	1 (1)
	Bronchiolitis	1 (1)	1 (1)	0 (0)	1 (1)
	Emphysema	2 (0)	2 (0)	0 (0)	0 (0)
	DAD	0 (1)	0 (0)	0 (1)	0 (1)
	Other	5 (3)	4 (2)	1 (1)	3 (1)
	Total	125 (86)	77 (54)	48 (32)	36 (29)

[0150] 125 samples (86 patients) were available for microarray classification. The 86 patients were randomized into training and test sets while controlling for patient-level pathology subtype bias (Table 1). The microarray training set consists of 77 samples (39 UIP and 38 non-UIP) from 54 patients. The microarray test set consists of 48 samples (22 UIP vs. 26 non-UIP) from 32 patients.

[0151] RNASeq data was generated for a subset of 36 samples (17 UIP and 19 non-UIP) from 29 patients (Table 1), representing a spectrum of ILD subtypes. Among the 36 samples, 22 overlap with the microarray training set and 14 overlap with the microarray test set. Due to the small sample size of this dataset, classification performance was evaluated by cross-validation (CV) only.

EXAMPLE 5

Training Models, Classification, Feature Selection

[0152] All statistical analyses were carried out using R version 3.0.1[21]. For the microarray classifier, genes differentially expressed between UIP and non-UIP classes were ranked by limma, then the top 200 genes with lowest false discovery rate (FDR) (< 0·0003) were carried forward as candidate genes for model building. Several models were built using different methods, and the one with the lowest error was chosen. Feature selection and model estimation were performed by logistic regression with *lasso* penalty using *glmnet*. For the RNASeq classifier, genes were ranked by FDR resulting from a Wald-style test implemented in the *DESeq2* package on the raw count data. The top features (N ranging from 10 to 200) were used to train a linear support vector machine (SVM) using the *e1071* library on the normalized expression data.

[0153] Classifier performance was evaluated by CV and, when available, by an independent test set. To minimize over-fitting, a single patient was maintained as the smallest unit when defining the training/test set and the CV partition; i.e. all samples belonging to the same patient were held together as a group in the training/test set or in CV partitions. The CV methods used include leave-one-patient-out (LOPO) and 10-fold patient-level CV.

[0154] Performance was reported as the area under the curve (AUC), and specificity (1·0 – false positive rate) and sensitivity (1·0 – false negative rate) at a given score threshold. We set the score threshold to require at least >90% specificity. For each performance measurement, 95% confidence intervals were computed using 2000 stratified bootstrap replicates and the *pROC* package and reported as [CI lower-upper].

EXAMPLE 6

Spatial Heterogeneity in Samplings From Explanted Lungs

[0155] A total of 60 samplings from three normal lung donors (n=7) and three lungs from patients diagnosed with IPF (n=53) were analyzed using genome-wide microarray data. Intact normal and diseased lungs obtained during transplant procedures were collected following a protocol approved by the Institutional Review Board (IRB) of Inova Fairfax, Falls Church, Virginia. The upper and lower lobes of explanted lungs from three normal donors and three patients diagnosed with IPF were sampled centrally and peripherally. The location and number of the explant samples is illustrated in Figure 6. Surgical pathology and final clinical diagnoses were provided by the originating institutions. Pathology over-reads by three expert pathologists unanimously confirmed UIP in all three IPF patient explant lungs.

[0156] Gene expression was evaluated in seven normal and 53 IPF explant lung samples. Genes differentially expressed between normal and IPF patient explant samples were identified and ranked by false discovery rate (FDR) using the R limma package (Smyth, G. K. (2005)). The top 200 genes differentially expressed between UIP and non-UIP classes in the microarray training set are shown in Table 12. Using the top 200 genes with the lowest FDR adjusted P-values (<1·45e-07), the Pearson correlation coefficient was calculated for all pairs of 53 UIP samples.

[0157] Table 12. Top 200 genes differentially expressed between UIP and non-UIP classes in the microarray training set, with indication of 22 genes used by the microarray classifier.

TCID	GENE SYMBOL	logFC	MedExpr.UIP	MedExpr.NonUIP	FDR adjusted p-value	rank	Used by Classifier
8117760	HLA-F	-0.48	9.02	9.53	2.35E-09	1	Used
8177717	HLA-F	-0.50	9.35	9.94	2.35E-09	2	
8179019	HLA-F	-0.50	9.31	9.83	2.35E-09	3	
8101031	CDKL2	-0.95	7.31	8.16	1.29E-07	4	Used
8106827	GPR98	-0.77	6.11	6.74	2.16E-07	5	Used
8100026	ATP8A1	-0.65	7.63	8.17	9.86E-07	6	
7931930	PRKCQ	-0.64	6.78	7.40	1.81E-06	7	Used
8135661	CFTR	-1.00	6.50	7.56	2.37E-06	8	
8177725	HLA-G	-0.29	10.67	10.89	2.37E-06	9	Used
8179034	HLA-G	-0.29	10.67	10.89	2.37E-06	10	
8123246	SLC22A3	-0.94	6.90	7.85	2.37E-06	11	
8118571	PSMB9	-0.53	10.04	10.68	2.37E-06	12	
8178211	PSMB9	-0.53	10.04	10.68	2.37E-06	13	
8179495	PSMB9	-0.53	10.04	10.68	2.37E-06	14	
8065719	PXMP4	-0.67	7.12	7.94	2.37E-06	15	
7926037	PFKFB3	-0.45	7.66	8.10	2.81E-06	16	Used
8037205	CEACAM1	-0.41	6.47	6.97	3.04E-06	17	Used

TCID	GENE SYMBOL	logFC	MedExpr.UIP	MedExpr.NonUIP	FDR adjusted p-value	rank	Used by Classifier
8178489	HLA-C	-0.36	11.26	11.64	3.04E-06	18	
7917561	GBP4	-0.87	8.49	9.57	3.59E-06	19	
7968678	FREM2	-1.08	6.67	7.72	3.88E-06	20	
7907492	RABGAP1L	-0.32	8.01	8.33	3.88E-06	21	Used
8124901	HLA-C	-0.35	11.27	11.61	3.89E-06	22	
8096682	ARHGEF38	-0.78	6.52	7.21	3.89E-06	23	
8049187	EFHD1	0.36	7.69	7.30	4.36E-06	24	
8117890	HLA-E	-0.28	10.64	10.89	4.54E-06	25	
8179731	HLA-B	-0.26	11.89	12.09	4.54E-06	26	
8154233	CD274	-0.84	6.35	7.14	4.54E-06	27	Used
8177788	HLA-E	-0.29	10.68	10.94	4.54E-06	28	
8179103	HLA-E	-0.29	10.68	10.94	4.54E-06	29	
8117777	HLA-H	-0.24	9.99	10.21	4.89E-06	30	
7981290	WARS	-0.55	9.39	9.97	5.08E-06	31	
8177732	HLA-A	-0.27	11.72	12.01	5.30E-06	32	
7965565	USP44	-0.71	5.92	6.68	5.30E-06	33	
8125512	TAP1	-0.55	8.54	9.14	5.30E-06	34	
8178867	TAP1	-0.55	8.54	9.14	5.30E-06	35	
8180061	TAP1	-0.55	8.54	9.14	5.30E-06	36	
8022145	L3MBTL4	-0.37	6.74	7.07	5.37E-06	37	
8106098	MAP1B	0.68	8.77	8.00	5.37E-06	38	
7934719	SFTPD	-0.71	9.02	9.63	5.75E-06	39	
7905929	EFNA1	-0.51	7.67	8.14	5.88E-06	40	
7917516	GBP1	-0.62	9.37	10.05	6.11E-06	41	
8161865	PRUNE2	0.79	7.44	6.75	7.05E-06	42	Used
8044353	ACOXL	-0.68	6.13	6.74	7.10E-06	43	
8057418	ZNF385B	-0.71	6.95	7.71	7.15E-06	44	
8101131	CXCL11	-1.44	5.33	6.99	8.16E-06	45	
8058498	FZD5	-0.52	7.72	8.27	8.87E-06	46	
8082100	PARP14	-0.36	8.93	9.25	9.55E-06	47	
8001007	PRSS8	-0.51	7.39	7.89	9.85E-06	48	
8099760	ARAP2	-0.41	7.55	7.90	1.06E-05	49	Used
7914950	CSF3R	-0.45	7.12	7.60	1.14E-05	50	
7972336	DZIP1	0.33	7.72	7.47	1.16E-05	51	Used
8014591	HNF1B	-0.53	6.88	7.33	1.20E-05	52	
8151423	JPH1	-0.52	6.68	7.17	1.21E-05	53	
8056217	MXRA7	0.40	8.58	8.29	1.21E-05	54	Used
8117861	HLA-L	-0.24	6.64	6.89	1.25E-05	55	
8179080	HLA-L	-0.24	6.64	6.89	1.25E-05	56	
7976443	IFI27	-0.57	9.21	9.74	1.54E-05	57	
8022022	LPIN2	-0.30	8.06	8.37	1.55E-05	58	
7997593	ATP2C2	-0.46	6.73	7.13	1.62E-05	59	
8054846	SCTR	-0.48	6.23	6.74	1.72E-05	60	
8178498	HLA-B	-0.26	11.86	12.07	1.87E-05	61	
8140971	SAMD9L	-0.48	8.09	8.52	2.12E-05	62	
7931728	LARP4B	-0.28	8.76	9.07	2.15E-05	63	
8058857	IGFBP5	0.62	10.41	9.73	2.15E-05	64	
7946504	TMEM41B	-0.39	7.58	7.95	2.61E-05	65	
8057744	STAT1	-0.60	9.64	10.45	2.72E-05	66	
8107129	SLCO4C1	-1.08	7.96	8.93	2.72E-05	67	
8109938	RANBP17	-0.58	6.10	6.71	2.72E-05	68	
7934271	PLA2G12B	-0.40	6.37	6.79	2.72E-05	69	
8126855	PTCHD4	0.31	6.51	6.22	2.72E-05	70	
8097829	FHDC1	-0.54	6.39	6.91	2.80E-05	71	
8140478	GSAP	-0.50	7.87	8.41	2.82E-05	72	
8079334	LIMD1	-0.34	7.37	7.66	2.83E-05	73	
7992828	IL32	-0.47	8.23	8.83	2.83E-05	74	

TCID	GENE SYMBOL	logFC	MedExpr.UIP	MedExpr.NonUIP	FDR adjusted p-value	rank	Used by Classifier
8103563	DDX60	-0.43	7.34	7.80	2.83E-05	75	
8082928	CLDN18	-1.10	8.77	9.84	2.83E-05	76	
7970716	LNX2	-0.46	8.40	8.79	3.01E-05	77	
7944739	CRTAM	-0.62	5.05	5.69	3.07E-05	78	
8089026	STX19	-0.49	5.91	6.36	3.18E-05	79	
8079377	CXCR6	-0.46	5.75	6.17	3.22E-05	80	
7956120	ERBB3	-0.65	7.37	7.96	3.67E-05	81	
7981514	AHNAK2	0.54	7.30	6.80	3.84E-05	82	
8134036	STEAP2	0.66	9.18	8.42	3.87E-05	83	
8109639	PTTG1	-0.67	7.91	8.53	3.89E-05	84	
8101118	CXCL9	-1.55	7.35	9.08	4.07E-05	85	
7919984	SELENBP1	-0.44	8.87	9.25	4.25E-05	86	
8108724	PCDHB10	0.61	5.78	5.12	4.27E-05	87	
8126853	PTCHD4	0.75	7.02	6.07	4.27E-05	88	Used
8134384	DYNC1I1	0.23	5.71	5.43	4.52E-05	89	
7935535	CRTAC1	-0.68	6.04	6.73	4.73E-05	90	
8097080	SYNPO2	0.89	9.20	8.26	4.73E-05	91	
8129410	THEMIS	-0.85	6.35	7.33	4.73E-05	92	
7968035	SPATA13	-0.24	6.85	7.12	4.83E-05	93	
8104022	PDLIM3	0.58	8.33	7.63	5.03E-05	94	Used
8125545	HLA-DOA	-0.48	8.31	8.98	5.03E-05	95	
8115147	CD74	-0.20	12.35	12.54	5.03E-05	96	
8144758	ZDHHC2	-0.31	7.85	8.21	5.03E-05	97	
7910466	CAPN9	-0.71	5.34	6.15	5.03E-05	98	
8124911	HLA-B	-0.25	11.87	12.05	5.12E-05	99	
7938834	NAV2	-0.36	7.79	8.04	5.18E-05	100	
8146092	IDO1	-1.21	6.59	8.00	5.43E-05	101	
8117800	HLA-A	-0.25	11.70	11.94	5.43E-05	102	
8025918	CNN1	0.86	8.39	7.52	5.43E-05	103	Used
8020847	DTNA	0.42	7.10	6.70	5.52E-05	104	
7934411	USP54	-0.43	7.89	8.31	5.61E-05	105	
8101126	CXCL10	-1.70	6.30	8.19	5.61E-05	106	
7993458	C16orf45	0.39	7.38	7.02	5.62E-05	107	
8157027	NIPSNAP3B	0.29	5.25	4.98	5.62E-05	108	Used
8007931	ITGB3	0.40	6.54	6.10	5.62E-05	109	
7947248	KIF18A	-0.48	5.00	5.51	6.16E-05	110	
7978360	GZMH	-0.58	6.95	7.54	6.51E-05	111	
8142997	PLXNA4	0.44	6.55	6.07	6.64E-05	112	
8125993	ETV7	-0.28	5.78	6.03	6.97E-05	113	
8149725	PEBP4	-1.05	8.77	9.75	7.36E-05	114	
8178295	UBD	-0.74	7.87	8.57	7.36E-05	115	
8122986	SNX9	0.28	8.54	8.22	7.50E-05	116	
8154981	UNC13B	-0.49	8.16	8.62	7.50E-05	117	
8175369	MAP7D3	0.55	8.76	8.26	8.13E-05	118	
8091600	PLCH1	-0.62	6.84	7.31	8.13E-05	119	
8124650	UBD	-0.75	7.99	8.74	8.22E-05	120	
8161044	TPM2	0.60	10.51	9.89	8.37E-05	121	
8002218	ESRP2	-0.41	7.21	7.56	8.37E-05	122	
8180093	HLA-DOA	-0.45	8.07	8.59	8.37E-05	123	
8136473	TRIM24	-0.28	8.33	8.58	8.41E-05	124	
7956856	MSRB3	0.44	7.99	7.55	8.49E-05	125	
8035304	BST2	-0.50	8.02	8.52	8.49E-05	126	
8072710	APOL6	-0.37	8.35	8.84	8.51E-05	127	
8052882	ADD2	0.28	5.63	5.38	8.58E-05	128	
7958019	DRAM1	-0.52	8.60	9.13	9.18E-05	129	

TCID	GENE SYMBOL	logFC	MedExpr.UIP	MedExpr.NonUIP	FDR adjusted p-value	rank	Used by Classifier
8069565	BTG3	-0.38	8.02	8.40	9.23E-05	130	
8114010	IRF1	-0.53	7.62	8.14	9.28E-05	131	
7986446	ALDH1A3	0.49	8.01	7.51	9.28E-05	132	
8068583	KCNJ15	-0.76	8.89	9.53	9.68E-05	133	
7909586	PPP2R5A	-0.37	7.92	8.32	1.05E-04	134	
8178220	HLA-DPB1	-0.54	9.76	10.34	1.06E-04	135	
8116932	PHACTR1	-0.38	7.38	7.81	1.15E-04	136	
8136095	AHCYL2	-0.57	8.98	9.39	1.23E-04	137	
8073088	APOBEC3G	-0.53	7.17	7.77	1.31E-04	138	
8109462	CNOT8	-0.26	8.54	8.78	1.33E-04	139	
8006608	CCL4L1	-0.64	6.44	7.12	1.39E-04	140	
8083709	SMC4	-0.29	8.48	8.78	1.41E-04	141	
8138489	CDCA7L	-0.44	7.79	8.21	1.45E-04	142	
7913858	PAQR7	0.22	6.58	6.38	1.46E-04	143	Used
8148070	COL14A1	0.71	9.87	9.29	1.48E-04	144	
8096314	PKD2	0.32	8.43	8.07	1.50E-04	145	
8014349	CCL15-CCL14	0.69	9.75	9.08	1.57E-04	146	
7919314	FMO5	-0.68	7.05	7.69	1.58E-04	147	
8006621	CCL4L1	-0.75	7.10	7.93	1.61E-04	148	
8019651	CCL4L1	-0.75	7.10	7.93	1.61E-04	149	
8089299	CD47	-0.25	10.44	10.72	1.61E-04	150	
7904106	MAGI3	-0.42	7.60	7.99	1.71E-04	151	
8008321	ACSF2	-0.34	6.38	6.73	1.75E-04	152	
8005048	MYOCD	0.92	7.04	6.16	1.77E-04	153	
8042788	ACTG2	0.98	10.11	9.21	1.79E-04	154	Used
7929466	CYP2C18	-0.35	5.09	5.39	1.84E-04	155	
8129888	NHSL1	-0.41	8.14	8.57	1.99E-04	156	
8173924	NA	-0.95	5.22	6.25	2.03E-04	157	Used
7923958	C1orf116	-0.74	8.38	9.00	2.07E-04	158	
7979269	GCH1	-0.42	6.93	7.36	2.16E-04	159	
8020495	CABLES1	-0.37	7.82	8.21	2.17E-04	160	
7919645	SV2A	0.28	6.06	5.82	2.18E-04	161	
8077458	EDEM1	-0.33	7.97	8.32	2.18E-04	162	
8117476	BTN3A3	-0.40	8.58	9.00	2.24E-04	163	
8021376	NEDD4L	-0.66	8.35	8.94	2.25E-04	164	
8056457	SCN1A	-0.52	4.80	5.29	2.25E-04	165	
8150962	TOX	-0.51	8.15	8.60	2.25E-04	166	
8058591	ACADL	-1.01	6.63	7.62	2.26E-04	167	
8126653	MRPL14	-0.37	9.31	9.62	2.29E-04	168	
8098611	TLR3	-0.44	8.52	8.89	2.29E-04	169	
8066822	SULF2	0.54	8.51	7.83	2.29E-04	170	
8109507	ITK	-0.60	6.14	6.97	2.30E-04	171	
8099506	TAPT1	-0.32	7.42	7.70	2.32E-04	172	
7973564	PSME1	-0.19	9.85	10.07	2.34E-04	173	
7897044	PRKCZ	-0.47	7.44	7.85	2.48E-04	174	
7974080	MIA2	-0.31	4.02	4.26	2.56E-04	175	
7917576	GBP5	-0.98	7.57	8.74	2.57E-04	176	
8085774	ZNF385D	0.99	7.66	6.39	2.58E-04	177	
7923386	LMOD1	0.69	7.68	7.05	2.58E-04	178	
8073522	SREBF2	-0.28	8.20	8.44	2.59E-04	179	

TCID	GENE SYMBOL	logFC	MedExpr.UIP	MedExpr.NonUIP	FDR adjusted p-value	rank	Used by Classifier
7981460	PPP1R13B	-0.25	7.83	8.04	2.62E-04	180	
8010454	RNF213	-0.36	8.63	9.01	2.63E-04	181	
8097903	TLR2	-0.37	7.77	8.26	2.68E-04	182	
8113369	SLCO4C1	-0.99	7.24	8.31	2.73E-04	183	
7950235	STARD10	-0.30	7.13	7.43	2.79E-04	184	
7910600	KIAA1804	-0.33	6.00	6.29	2.79E-04	185	
8117435	BTN3A2	-0.52	9.06	9.56	2.79E-04	186	
8143327	PARP12	-0.30	7.96	8.30	2.80E-04	187	
8087925	TNNC1	-0.90	8.53	9.30	2.82E-04	188	
8022045	MYOM1	0.42	5.80	5.42	2.82E-04	189	
8096070	BMP3	-0.60	7.22	7.81	2.82E-04	190	
8075709	APOL4	-0.39	7.01	7.41	2.87E-04	191	
7915500	C1orf210	-0.32	7.17	7.59	2.91E-04	192	
7920297	S100A14	-0.56	8.42	8.87	2.94E-04	193	
7983630	FGF7	0.67	7.64	6.98	2.94E-04	194	
8010287	C1QTNF1	0.35	7.81	7.49	3.09E-04	195	
8018966	TIMP2	0.18	10.53	10.34	3.09E-04	196	Used
7951593	NA	-0.51	7.28	7.84	3.16E-04	197	
8048541	DES	0.71	8.81	8.19	3.20E-04	198	Used
7975361	KIAA0247	-0.22	8.17	8.37	3.20E-04	199	
8170428	MTM1	-0.31	7.23	7.52	3.22E-04	200	

Abbreviations: TCID = transcript-cluster identity; Symbol = gene symbol; logFC = log fold-change; MedExpr.UIP = median expression level across the UIP samples; MedExpr.NonUIP = median expression level across the UIP samples; FDR = false discovery rate; Used by classifier = indicator of whether the gene is used by the microarray classifier.

[0158] The number and location of the samplings (upper vs. lower and central vs. peripheral) are indicated in Figure 6 and IPF patient clinical characteristics in Table 4. To identify genes useful in measuring spatial heterogeneity, we looked for differential expression in normal versus IPF samples. This comparison produced ~5,000 significantly differentially expressed RNA transcripts with FDR < 0·05 (data not shown). We selected the top 200 differentially expressed genes and measured pairwise correlation. The results for the three patients diagnosed with IPF are shown in Figure 1. Although correlation across all IPF samples is high, three distinct patterns emerge in the correlation structure among IPF samples. One patient (P1) shows substantial differences in upper vs. lower lobe gene expression i.e. lower correlation in gene signals. One patient (P3) shows higher correlation between the upper and lower lobe samplings. The third patient (P2) shows an intermediate result between these two cases, with sometimes higher, and sometimes lower, correlation between samplings from the upper and lower lobes. These results, while on a small number of patients, suggest that samples with lobe-specific pathology may be more accurate during the training phase of classifier development. Based on this information we prepared a classifier using SLB tissues

with truth labels assigned at the sample level, using lobe-derived pathology. Our results, which are presented in Example 7, demonstrate the presence of a molecular signature in SLB tissues that classifies UIP and non-UIP samples with high prospective accuracy.

[0159] Table 4. Clinical characteristics of three IPF explant patients.

Patient	Gender	Age	Smoking Status	Clinical Remarks
P1	M	49	non-smoker	Exertional dyspnea for ~2 years prior to initial evaluation. Pre-transplant SLB demonstrated clear UIP pattern, no granuloma or bronchiolocentricity. Diagnosis of IPF at transplant reported patchy subpleural and paraseptal interstitial fibrosis, dense scarring and honeycombing by surgical pathology. No evidence of granuloma or extensive inflammation.
P2	M	68	50 pack years	Initial evaluation occurred almost immediately after first presentation of exertional dyspnea; progressive worsening over ~2.5 years. Pathology at transplant demonstrated end-stage lung disease, diffuse fibrosis, temporal heterogeneity and fibroblastic foci suggestive of UIP.
P3	F	64	24 pack years	Exertional dyspnea for ~2 years prior to initial evaluation, worsening over the last year. Possible occupational exposure. Pre-transplant SLB demonstrated UIP pattern with fibroblastic foci consistent with IPF. Pathology at transplant showed interstitial fibrosis, giant cell reaction, reorganization, bronchiectasis and reactive lymph node.

EXAMPLE 7

Performance of Microarray Classifier on Surgical Lung Biopsies

[0160] Using sample-specific pathology labels on biopsies obtained during VATS, a microarray classifier was trained by logistic regression on the top 200 genes separating UIP and non-UIP samples (see Table 12). A final model was built with 22 genes (Table 5).

[0161] Expression data was normalized by Robust Multi-array Average (RMA). Feature selection and model estimation were performed by logistic regression with lasso penalty using glmnet3. Raw reads were aligned using TopHat. Gene counts were obtained using HTSeq and normalized using DESeq. The top features (N ranging from 10 to 200) were used to train a linear support vector machine (SVM) using the e1071 library. Confidence intervals were computed using the pROC package.

[0162] LOPO CV performance is summarized as a receiver operating characteristic (ROC) curve (Figure 2A). The AUC is 0·9 [CI 0·82-0·96], with 92% [CI 84%-100%] specificity and 64% [CI 49%-79%] sensitivity. Individual LOPO CV classification scores are shown for all patients (Figure 2B). Among the three misclassified non-UIP samples, two have scores very close to the threshold (0·86 and 1·30), and one has a high score (4·21). The latter sample with the high score was diagnosed as an 'unclassifiable fibrotic ILD' at both the sample- and patient-level. Among the UIP samples, fifteen (36%) have a score below the threshold (false negatives) but none of those samples have a large negative score. Since LOPO CV in certain cases has the potential to overestimate performance, we also evaluated 10-fold patient-level CV (i.e., 10% of patients are left out in each loop) which gives very similar performance (the median AUC from five repeated 10-fold CVs is 0·88).

[0163] Table 5. Twenty two genes included in a preferred array classifier.

TCID	SYMBOL (SEQ ID NO.)	logFC	MedExpr.UIP	MedExpr.NonUIP	FDR
8117760	HLA-F (1)	-0.48	9.02	9.53	2.35E-09
8101031	CDKL2 (2)	-0.95	7.31	8.16	1.29E-07
8106827	GPR98 (3)	-0.77	6.11	6.74	2.16E-07
7931930	PRKCQ (4)	-0.64	6.78	7.4	1.81E-06
8177725	HLA-G (5)	-0.29	10.67	10.89	2.37E-06
7926037	PFKFB3	-0.45	7.66	8.1	2.81E-06

	(6)				
8037205	CEACAM1	-0.41	6.47	6.97	3.04E-06
	(7)				
7907492	RABGAP1L	-0.32	8.01	8.33	3.88E-06
	(8)				
8154233	CD274	-0.84	6.35	7.14	4.54E-06
	(9)				
8161865	PRUNE2	0.79	7.44	6.75	7.05E-06
	(10)				
8099760	ARAP2	-0.41	7.55	7.9	1.06E-05
	(11)				
7972336	DZIP1	0.33	7.72	7.47	1.16E-05
	(12)				
8056217	MXRA7	0.4	8.58	8.29	1.21E-05
	(13)				
8126853	PTCHD4	0.75	7.02	6.07	4.27E-05
	(14)				
8104022	PDLIM3	0.58	8.33	7.63	5.03E-05
	(15)				
8025918	CNN1	0.86	8.39	7.52	5.43E-05
	(16)				
8157027	NIPSNAP3B	0.29	5.25	4.98	5.62E-05
	(17)				
7913858	PAQR7	0.22	6.58	6.38	1.46E-04
	(18)				
8042788	ACTG2	0.98	10.11	9.21	1.79E-04
	(19)				
8173924	NA	-0.95	5.22	6.25	2.03E-04
	(20)				
8018966	TIMP2	0.18	10.53	10.34	3.09E-04
	(21)				
8048541	DES	0.71	8.81	8.19	3.20E-04
	(22)				

[0164] Abbreviations: TCID = transcript-cluster identity; Symbol = gene symbol; logFC = log fold-change; MedExpr.UIP = median expression level across the UIP samples; MedExpr.NonUIP = median expression level across the UIP samples; FDR = false discovery rate.

[0165] Independent test set performance is shown in Figure 2C showing an AUC of 0·94 [CI 0·86-0·99], with 92% [CI 81%-100%] specificity and 82% [CI 64%-95%] sensitivity. The individual classification score distribution shows good separation between UIP and non-UIP classes (Figure 2D). The two misclassified non-UIP samples have both patient- and sample-level expert diagnoses of 'unclassifiable fibrotic ILD' indicating uncertainty in the diagnosis. The score range observed in the test set (Figure 2D) is narrower than the range seen in LOPO

CV scores (Figure 2B), likely due to the larger variability inherent in applying a series of sub-classifiers within each CV loop, compared to scores obtained by applying a single model. Classification performance including 95% confidence intervals are summarized in Table 6.

[0166] Table 6. Classifier performance summary, including 95% confidence intervals (CI).

	Array classifier (LOPO CV)	**Array classifier (Testing)**	**RNASeq classifier (LOPO CV)**	**RNASeqSet-matched array classifier (LOPO CV)**
AUC [95% CI]	0.90 [0.82-0.96]	0.94 [0.86-0.99]	0.90 [0.77-1.00]	0.86 [0.73-0.96]
Specificity [95% CI]	92% [84%-100%]	92% [81%-100%]	95% [84%-100%]	95% [84%-100%]
Sensitivity [95% CI]	64% [49%-79%]	82% [64%-95%]	59% [35%-82%]	47% [24%-71%]
Threshold	0.72	1.04	0.64	1
False positive count	3	2	1	1
True negative count	35	24	18	18
False negative count	14	4	7	9
True negative count	25	18	10	8
Total	77	48	36	36

[0167] Our approach offers significant advantages. Earlier gene-expression profiling studies focused on comparing IPF versus a few non-IPF ILD subtypes such as HP or NSIP, or against subjects without ILD[18,19,23,25]. The non-UIP cohort reported here represents a broad spectrum of pathology subtypes including HP, NSIP, sarcoidosis, RB, bronchiolitis, organizing pneumonia (OP), and others, thus approximating the diversity of ILDs encountered in clinical practice. In addition, the classifier was trained and tested using a combination of banked and prospectively collected SLBs to ensure robustness against potential differences in sample handling and collection. Finally, many earlier studies focused on differential gene expression analyses alone, without building a classification engine. In contrast, our approach is a rigorous method for the development of molecular tests which, when properly trained and validated, generalized well to independent data sets.

EXAMPLE 8

Performance of RNASeq classifier on surgical lung biopsies

[0168] A subset of 36 samples with RNASeq data were used to train a linear SVM classifier and the performance evaluated by LOPO CV. AUCs are consistently above 0·80 for gene numbers spanning 10 to 200 (data not shown). We chose a model using 100 genes for further examination. The AUC is 0·9 [CI 0·77-1·00] (specificity=95% [CI 84%-100%], sensitivity=59% [CI 35%-82%]) (Figure 3A). Only a single non-UIP sample is misclassified (Figure 3B). The sample-level pathology for this sample is respiratory bronchiolitis (RB), and the patient-pathology is diffuse alveolar damage (DAD), two subtypes that may have been difficult to model because of their sparsity. We carried out a similar analysis using matching array data on the same set of samples; the array-based classifier achieves similar performance (AUC=0·86 [CI 0·73-0·96]) using 160 genes. Specificity is 95% [CI 84%-100%] and sensitivity is 47% [CI 24%-71%] (Figure 3C). Interestingly, the same non-UIP sample that was misclassified as a UIP by the RNASeq classifier is also misclassified by the microarray classifier (Figure 3D). Overall, classification based on RNASeq achieves comparable performance to that of the array platform.

EXAMPLE 9

Biological Pathways Associated With Genes Used By The Classifiers

[0169] To determine if there are common biological underpinnings across the genes selected by the machine learning process, we used over-representation analysis (ORA) to identify statistically significant participation of genes in selected pathways. Over/under-representation analyses (ORA) were performed using GeneTrail software (genetrail.bioinf.uni-sb.de/) and the top 1,000 genes differentially expressed by limma between UIP and non-UIP samples (FDR < 0·013) in the microarray testing set (n=77) as the ORA test sets. The ORA reference set included all human genes (n=44,829) and annotation in the KEGG pathways and gene ontology (GO) databases. Significance was evaluated via Fisher's exact test with a corrected FDR threshold of $p < 0·05$.

[0170] In examining the top 1000 genes found in the UIP vs non-UIP comparison, distinct findings emerged (Table 2).

[0171] **Table 2**. Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways and Gene Ontologies (GO) over-represented in UIP and non-UIP samples. Categories in each sample cohort are ranked by FDR p value.

Over-represented in UIP

Source	Category	No. expected	No. observed	ORA Proportion	FDR p value
GO	Extracellular matrix	5	31	6.2	1.46E-12
GO	Muscle system process	5	23	4.6	1.21E-07
GO	Cell migration/motility	9	27	3.0	7.31E-06
KEGG	Focal adhesion	6	21	3.5	1.20E-05
GO	Contractile fiber	2	12	6.0	1.73E-05
KEGG	Calcium signaling, pathway	5	18	3.6	5.38E-05
KEGG	Dilated cardiomyopathy	3	13	4.3	5.38E-05
KEGG	ECM-receptor interaction	2	12	6.0	5.95E-05
KEGG	Vascular Muscle contraction	3	14	4.7	5.95E-05
KEGG	Hypertropic cardiomyopathy (HCM)	2	11	5.5	2.90E-04
KEGG	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	2	9	4.5	2.82E-03
KEGG	Regulation of Actin cytoskeleton	6	16	2.7	3.39E-03
KEGG	Melanoma	2	8	4.0	6.26E-03
KEGG	Cardiac muscle contraction	2	7	3.5	4.53E-02
KEGG	MAPK signaling pathway	8	15	1.9	4.76E-02

Over-represented in non-UIP

Source	Category	No. expected	No. observed	ORA Proportion	FDR p-value
KEGG	Allograft rejection	1	15	15.0	9.25E-12
KEGG	Cell adhesion molecules	4	25	6.3	9.25E-12
KEGG	Antigen processing and presentation	2	19	9.5	3.40E-11
KEGG	Type I diabetes mellitus	1	14	14.0	5.95E-10
GO	Immune response	13	42	3.2	6.52E-09
KEGG	Autoimmune thyroid disorder	2	14	7.0	6.72E-09
KEGG	Viral myocarditis	2	16	8.0	6.72E-09
KEGG	Phagosome	5	20	4.0	7.01E-07
GO	MHC class I receptor activity	1	7	7.0	2.66E-05
KEGG	Systemic lupus erythematosus	4	16	4.0	6.11E-05
KEGG	Leishmaniasis	2	11	5.5	1.14E-04
GO	Innate immune response	3	14	4.7	2.34E-04
KEGG	Asthma	1	7	7.0	2.83E-04
GO	Signal transducer activity	24	48	2	3.44E-04
GO	Antigen processing and presentation of endogenous antigen	1	4	4	7.50E-04
KEGG	Intestinal immune network for IgA production	2	8	4.0	8.40E-04
KEGG	Malaria	2	8	4.0	1.04E-03
GO	T cell activation	3	12	4.0	1.58E-03
KEGG	Natural killer cell mediated cytotoxicity	4	13	3.3	1.91E-03
KEGG	Endocytosis	6	16	2.7	3.42E-03
KEGG	Steroid biosynthesis	1	4	4.0	8.78E-03

KEGG	Tight junction	4	11	2.8	1.50E-02
KEGG	Proteasome	1	6	6.0	1.71E-02
KEGG	Primary immunodeficiency	1	5	5.0	1.89E-02
KEGG	Toll-like receptor signaling	3	9	3.0	1.89E-02

[0172] Abbreviations: FDR = false discovery rate; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; ORA = over-representation analysis.

[0173] In UIP, genes involved in cell adhesion, muscle disease, cell migration and motility predominate. These results are consistent with previous reports of pathways differentially regulated in IPF[18,19,22,23]. In contrast, other non-UIP subtypes overexpress genes involved in immune processes, including both the adaptive and innate systems. This enrichment could be due to the RB and HP subtypes present in the non-UIP cohort; diseases known to exhibit immune components[24]. Genes over-represented in KEGG pathways and Gene Ontology groups are summarized in Tables 7 and 8.

[0174] Table 7. Genes over-represented in KEGG pathways and Gene Ontology groups of UIP samples.

OVER-REPRESENTED IN UIP			
Source	**Category**	**No. observed**	**Genes Observed**
GO	Extracellular matrix	31	ABI3BP, ADAMTS3, AEBP1, CLU, COL14A1, COL15A1, COL1A2, COL21A1, COL6A1, COL6A2, CPXM2, DST, FBLN1, FBN1, FGF10, FMOD, HTRA1, LAMA4, LAMB2, LAMC1, LTBP1, MGP, NID1, PLAT, POSTN, SERPINF1, SFRP2, SNCA, SPON1, TNC, VCAN
GO	Muscle system process	23	ACTA2, ACTG2, AGT, ATP1A2, BDKRB2, CALD1, CNN1, CRYAB, DES, DTNA, IGF1, LMOD1, MYH11, MYL9, MYLK, MYOCD, SLC6A8, SSPN, TNNI2, TNNT3, TPM1, TPM2, TPM4
GO	Cell migration/motility	27	ADRA2A, AGT, ANGPT2, CCL24, CXCL12, ENPP2, F10, FGF10, FGF7, IGF1, IGFBP5, ITGB3, KRT2, LAMC1, NEXN, NR2F2, PDGFRB, PODN, PPAP2A, ROR2, S100A2, SCG2, SFRP2, SLIT3, THY1, TPM1, VCAN
KEGG	Focal adhesion	21	ACTN1, COL1A2, COL6A1, COL6A2, FLNC, HGF, IGF1, ITGA7, ITGB3, ITGB4, LAMA4, LAMB2, LAMC1, MYL9, MYLK, PARVA, PDGFD, PDGFRB, SHC4, SPP1, TNC
GO	Contractile fiber	12	DES, MYH11, MYL9, MYOM1, NEXN, PGM5, SVIL, TNNI2, TNNT3, TPM1, TPM2, TPM4

KEGG	Calcium signaling, pathway	18	ADCY3, AVPR1A, BDKRB2, CACNA1C, GNAL, GRIN2A, HRH1, HTR2A, MYLK, P2RX1, PDE1A, PDGFRB, PLCB1, PLN, PTGER3, RYR3, TACR1, TRPC1
KEGG	Dilated cardiomyopathy	13	ADCY3, ADCY5, CACNA1C, CACNA2D1, DES, IGF1, ITGA7, ITGB3, ITGB4, PLN, TPM1, TPM2, TPM4
KEGG	ECM-receptor interaction	12	COL1A2, COL6A1, COL6A2, ITGA7, ITGB3, ITGB4, LAMA4, LAMB2, LAMC1, SPP1, SV2A, TNC
KEGG	Vascular Muscle contraction	14	ACTA2, ACTG2, ADCY3, ADCY5, AVPR1A, CACNA1C, CALD1, KCNMB1, MRVI1, MYH11, MYL9, MYLK, PLA2G2A, PLCB1,
KEGG	Hypertropic cardiomyopathy (HCM)	11	CACNA1C, CACNA2D1, DES, IGF1, ITGA7, ITGB3, ITGB4, PRKAA2, TPM1, TPM2, TPM4
KEGG	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	9	ACTN1, CACNA1C, CACNA2D1, CDH2, DES, ITGA7, ITGB3, ITGB4, PKP2
KEGG	Regulation of Actin cytoskeleton	16	ACTN1, BDKRB2, ENAH, FGF10, FGF14, FGF7, FGFR1, GNG12, ITGA7, ITGB3, ITGB4, MRAS, MYL9, MYLK, PDGFD, PDGFRB
KEGG	Melanoma	8	FGF10, FGF14, FGF7, FGFR1, HGF, IGF1, PDGFD, PDGFRB
KEGG	Cardiac muscle contraction	7	ATP1A2, ATP1B2, CACNA1C, CACNA2D1, TPM1, TPM2, TPM4
KEGG	MAPK signaling pathway	15	CACNA1C, CACNA2D1, FGF10, FGF14, FGF7, FGFR1, FLNC, GNG12, HSPA2, MAP4K4, MRAS, NFATC4, PDGFRB, PLA2G2A, ZAK

[0175] Table 8. Genes over-represented in KEGG pathways and Gene Ontology groups of non-UIP samples.

OVER-REPRESENTED IN non-UIP			
Source	**Category**	**No. observed**	**Genes Observed**
KEGG	Allograft rejection	15	CD40, FASLG, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, IFNG
KEGG	Cell adhesion molecules	25	CADM1, CD2, CD274, CD40, CD8A, CLDN18, CLDN4, F11R, HLA-A, HLA-B, HLA-C, HLA-, DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, ICAM1, ICOS, ITGAL, OCLN, SDC4
KEGG	Antigen processing and presentation	19	CD74, CD8A, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-, DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, IFNG, PSME1, PSME2, TAP1, TAP2
KEGG	Type I diabetes mellitus	14	FASLG, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-, DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, IFNG
GO	Immune response	42	APOBEC3G, AQP4, BST2, C2, CADM1, CCR5, CD274, CD74, CD8A, CRTAM, CTSC, CTSW, CXCL16, ERAP1, FCGR1A, FCGR1B, FCGR3A, GBP2, GCH1, GZMA, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DQA1, HLA-DRA, HLA-H, ICAM1, ICOS, IL32, ITGAL, MICB, NUB1, PLA2G1B, PSMB10, S100A14, SFTPD, SKAP1, THEMIS, TLR2, TLR3, UBD
KEGG	Autoimmune thyroid disorder	14	CD40, FASLG, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G
KEGG	Viral myocarditis	16	CD40, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, ICAM1, ITGAL, MYH14
KEGG	Phagosome	20	ATP6V0D1, FCGR1A, FCGR3A, HLA-A, HLA-B, HLA-C, HLA-DMA, HLA-DOA, HLA-, DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, SFTPA1, SFTPD, TAP1, TAP2, TLR2
GO	MHC class I receptor activity	7	HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H

OVER-REPRESENTED IN non-UIP			
Source	**Category**	**No. observed**	**Genes Observed**
KEGG	Systemic lupus erythematosus	16	C2, CD40, FCGR1A, FCGR3A, HIST1H2BJ, HIST1H3F, HIST1H4E, HIST2H2BE, HLA-, DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, IFNG, TRIM21
KEGG	Leishmaniasis	11	FCGR1A, FCGR3A, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-, DRA, IFNG, STAT1, TLR2
GO	Innate immune response	14	APOBEC3G, AQP4, C2, CADM1, CRTAM, CXCL16, ERAP1, GCH1, NUB1, S100A14, SFTPD, TLR2, TLR3, UBD
KEGG	Asthma	7	CD40, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA
GO	Signal transducer activity	48	AGER, BST2, CCL4, CCL5, CCR5, CD2, CD40, CD47, CD74, CD8A, CLDN4, CXCL16, CXCR6, EBP, ERBB3, FCGR1A, FCGR1B, FGFR2, FLRT3, FZD5, GPR98, HLA-A, HLA-B, HLA-C, HLA-DOA, HLA-DPA1, HLA-DQA1, HLA-DRA, HLA-E, HLA-F, HLA-G, HLA-H, ICAM1, IL2RB, KLRB1, LDLR, MAP3K13, PTPRJ, SCTR, SDC4, SH2D1A, SKAP1, STAT1, TAPT1, TLR2, TLR3, TP53BP2, UNC13B
GO	Antigen processing and presentation of endogenous antigen	4	CD74, ERAP1, TAP1, TAP2
KEGG	Intestinal immune network for IgA production	8	CD40, HLA-DMA, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, ICOS
KEGG	Malaria	8	CD40, ICAM1, IFNG, ITGAL, KLRB1, KLRK1, SDC4, TLR2
GO	T cell activation	12	CADM1, CD2, CD47, CD74, CD8A, CRTAM, ICAM1, ITGAL, KLRK1, MICB, SFTPD, THEMIS
KEGG	Natural killer cell mediated cytotoxicity	13	FASLG, FCGR3A, HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, ICAM1, IFNG, ITGAL, KLRK1, MICB, SH2D1A
KEGG	Endocytosis	16	ARAP2, CCR5, DNM2, ERBB3, FGFR2, HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, IL2RB, LDLR, NEDD4L, PARD6B, PRKCZ
KEGG	Steroid biosynthesis	4	DHCR24, EBP, FDFT1, SQLE
KEGG	Tight junction	11	CLDN18, CLDN4, F11R, INADL, LLGL2, MAGI3, MYH14, OCLN, PARD6B, PRKCQ, PRKCZ
KEGG	Proteasome	6	IFNG, PSMB10, PSMB8, PSMB9, PSME1, PSME2
KEGG	Primary	5	CD40, CD8A, ICOS, TAP1, TAP2

OVER-REPRESENTED IN non-UIP			
Source	**Category**	**No. observed**	**Genes Observed**
KEGG	immunodeficiency Toll-like receptor signaling	9	CCL4, CCL5, CD40, CXCL10, CXCL11, CXCL9, STAT1, TLR2, TLR3

EXAMPLE 10

Mislabeling Simulation Study

[0176] A simulation study swapping binary classification labels (UIP or non-UIP) was performed on the microarray training set. Samples were selected at random for label permutation, at total proportions per simulation set ranging from 1% to 40%. The level of agreement in the blinded review of the three expert pathology diagnoses is 3/3 (n=44), 2/2 (n=8), 2/3 (n=24), and 1/3 (n=1). Sample labels were changed to the other class with a weight proportional to the probability accounting for the disagreement level in the blinded review of the three expert pathologists: 5% for 3/3 or 2/2 agreement, 50% for 2/3 agreement, and 90% for 1/3 agreement. Simulations were repeated 100 times at each proportion.

[0177] The LOPO CV performance (AUC) was evaluated over 100 repeated simulations across a range of proportion of swapped labels (Figure 4). When there is no label swap, the median performance is very close to the array classifier performance shown in Figure 2A (AUC=0·9). (Using the same set of samples and labels, model estimation can have slight variability). As the swap rate increases, the performance decreases monotonically. When 40% of the labels are swapped, the median performance approaches 0·5, indicating classification is no better than random chance.

EXAMPLE 11

Magnitude and direction of UIP/Non-UIP differential gene expression differs in smoker vs. non-smoker test subjects.

[0178] Interstitial lung diseases are more prevalent in persons that smoke, or have had a long history of smoking prior to quitting, than in persons who never smoked. We compared differential gene expression profiles of samples derived from smoker and non-smoker UIP or non-UIP subjects to determine if smoking status affects performance of UIP diagnostic classifiers.

[0179] Transbronchial biopsy samples were prepared [according to the methods described in Examples 1 and 2, and RNA sequencing analysis was performed according to the method described in Example 3]. RNASeq data was generated for a subset of 24 samples (9 UIP and 15 non-UIP), and differential gene expression was analyzed according to three binary comparisons: (i) UIP vs. non-UIP, n=9 and 15 samples, respectively; (ii) Non-smoker UIP vs. Non-smoker non-UIP, n=3 and 5 samples, respectively; and (iii) Smoker UIP vs. Smoker non-UIP, n=12 and 4 samples, respectively.

[0180] The results of the expression analysis for groups (i) to (iii) are shown in Tables 9 to 11, respectively, and are summarized in Figures 8-10. The number of genes differentially expressed between UIP and Non UIP samples differs drastically between smokers and non-smokers (64 differentially expressed in samples from smokers, 671 differentially expressed in samples from non-smokers) (Figure 8). Moreover, certain genes that were differentially upregulated in non-smokers were downregulated on not differentially expressed in smokers (Figures 9 and 10). These data demonstrate that certain genes that are useful in UIP classification of samples from non-smokers are not informative, or can be contradictory in the diagnosis of the same disease, in smokers. Smoker-status differences in gene expression can reduce the performance of gene expression classifier predictions generated using traditional 2-class machine learning methods. We overcome this problem using three different techniques that are optionally combined or used individually in combination with the UIP vs. Non-UIP classifiers and methods of diagnosis UIP vs. Non-UIP via the diagnostic methods disclosed herein.

[0181] In a first approach, smoking status (smoker vs. non-smoker) is used as a covariate in the model during training. This simple approach boosts signal-to-noise ratio, particularly in data derived from smokers (were noise is higher) and allows data derived from smokers and non-smokers to be combined and used simultaneously.

[0182] In a second approach, genes that are susceptible to smoker-status bias are identified and excluded, or optionally weighted differently than genes that are not susceptible to such bias, during classifier training. This method enriches the feature space used for training with genes that are not confounded or affected by smoking status.

[0183] In a third approach, a tiered classification effort is utilized in which an initial classifier is trained to recognize gene signatures that distinguish smokers from non-smokers. Once

patient samples are pre-classified as "smoker" or "non-smoker", distinct classifiers that were each trained to distinguish UIP vs. Non UIP in smokers or non-smokers, respectively, are implemented. Such smoker or non-smoker-specific classifiers provide improved diagnostic performance.

[0184] Table 9. Differentially expressed genes in UIP vs. Non-UIP samples, irrespective of smoker status.

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000204256	6046	BRD2	-0.30	4.03E-11	6.40E-07
ENSG00000129204	9098	USP6	1.88	2.57E-10	2.04E-06
ENSG00000148357	256158	HMCN2	1.71	1.62E-08	8.58E-05
ENSG00000178031	92949	ADAMTSL1	1.69	1.23E-07	4.83E-04
ENSG00000112130	9025	RNF8	-0.40	1.52E-07	4.83E-04
ENSG00000197705	57565	KLHL14	1.58	1.97E-07	5.21E-04
ENSG00000204632	3135	HLA-G	-1.65	4.51E-07	1.02E-03
ENSG00000157064	23057	NMNAT2	1.47	6.62E-07	1.31E-03
ENSG00000112245	7803	PTP4A1	-0.59	1.38E-06	2.43E-03
ENSG00000114115	5947	RBP1	0.95	1.69E-06	2.68E-03
ENSG00000152413	9456	HOMER1	-0.48	2.30E-06	3.32E-03
ENSG00000143603	3782	KCNN3	0.81	2.85E-06	3.54E-03
ENSG00000198074	57016	AKR1B10	-1.48	2.90E-06	3.54E-03
ENSG00000160007	2909	ARHGAP35	-0.28	4.24E-06	4.49E-03
ENSG00000076053	10179	RBM7	-0.30	4.23E-06	4.49E-03
ENSG00000085563	5243	ABCB1	1.40	5.90E-06	5.55E-03
ENSG00000047365	116984	ARAP2	-0.60	5.95E-06	5.55E-03
ENSG00000130600	283120	H19	1.45	7.10E-06	5.68E-03
ENSG00000131355	84658	ADGRE3	1.43	7.22E-06	5.68E-03

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000135837	9857	CEP350	-0.23	6.63E-06	5.68E-03
ENSG00000102221	9767	JADE3	-0.42	7.52E-06	5.68E-03
ENSG00000105784	154661	RUNDC3B	1.31	1.02E-05	6.94E-03
ENSG00000198734	2153	F5	0.96	1.05E-05	6.94E-03
ENSG00000124226	55905	RNF114	-0.39	1.04E-05	6.94E-03
ENSG00000106852	26468	LHX6	1.16	1.12E-05	7.10E-03
ENSG00000183454	2903	GRIN2A	1.41	1.47E-05	8.93E-03
ENSG00000170153	57484	RNF150	1.04	1.54E-05	8.93E-03
ENSG00000187527	344905	ATP13A5	-1.39	1.58E-05	8.93E-03
ENSG00000244734	3043	HBB	1.40	1.71E-05	9.05E-03
ENSG00000204936	57126	CD177	1.40	1.67E-05	9.05E-03
ENSG00000136560	10010	TANK	-0.37	2.26E-05	1.16E-02
ENSG00000196562	55959	SULF2	0.98	2.87E-05	1.42E-02
ENSG00000151789	79750	ZNF385D	1.36	3.17E-05	1.44E-02
ENSG00000145808	171019	ADAMTS19	1.07	3.14E-05	1.44E-02
ENSG00000177666	57104	PNPLA2	-0.53	3.00E-05	1.44E-02
ENSG00000007312	974	CD79B	1.31	3.51E-05	1.47E-02
ENSG00000104213	5157	PDGFRL	1.24	3.53E-05	1.47E-02
ENSG00000023171	57476	GRAMD1B	-0.86	3.41E-05	1.47E-02
ENSG00000117322	1380	CR2	1.31	4.37E-05	1.78E-02
ENSG00000171724	57687	VAT1L	1.33	4.51E-05	1.79E-02
ENSG00000108852	4355	MPP2	1.21	4.84E-05	1.87E-02
ENSG00000136098	4752	NEK3	0.51	5.00E-05	1.89E-02
ENSG00000189056	5649	RELN	1.29	5.13E-05	1.89E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000054938	25884	CHRDL2	1.30	6.70E-05	2.36E-02
ENSG00000132704	79368	FCRL2	1.30	6.62E-05	2.36E-02
ENSG00000196628	6925	TCF4	0.50	7.00E-05	2.41E-02
ENSG00000142856	23421	ITGB3BP	0.55	7.32E-05	2.47E-02
ENSG00000136153	4008	LMO7	-0.80	8.61E-05	2.85E-02
ENSG00000203867	282996	RBM20	1.20	9.01E-05	2.92E-02
ENSG00000171049	2358	FPR2	1.26	9.26E-05	2.94E-02
ENSG00000152953	55351	STK32B	1.23	9.47E-05	2.95E-02
ENSG00000099968	23786	BCL2L13	-0.37	9.73E-05	2.97E-02
ENSG00000188536	3040	HBA2	1.26	1.03E-04	3.00E-02
ENSG00000198569	142680	SLC34A3	0.94	1.04E-04	3.00E-02
ENSG00000163701	132014	IL17RE	-0.60	1.02E-04	3.00E-02
ENSG00000197614	8076	MFAP5	1.25	1.10E-04	3.12E-02
ENSG00000146021	26249	KLHL3	0.84	1.15E-04	3.19E-02
ENSG00000156103	4325	MMP16	1.25	1.21E-04	3.24E-02
ENSG00000185022	23764	MAFF	-1.05	1.20E-04	3.24E-02
ENSG00000088882	56265	CPXM1	1.22	1.32E-04	3.49E-02
ENSG00000163032	7447	VSNL1	0.78	1.38E-04	3.58E-02
ENSG00000186184	51082	POLR1D	-0.47	1.40E-04	3.58E-02
ENSG00000177106	64787	EPS8L2	-0.52	1.42E-04	3.58E-02
ENSG00000214711	440854	CAPN14	1.23	1.44E-04	3.58E-02
ENSG00000113212	56129	PCDHB7	1.04	1.51E-04	3.64E-02
ENSG00000001629	54467	ANKIB1	-0.22	1.52E-04	3.64E-02
ENSG00000180871	3579	CXCR2	1.20	1.79E-04	4.17E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000065618	1308	COL17A1	1.04	1.79E-04	4.17E-02
ENSG00000008394	4257	MGST1	-0.59	1.81E-04	4.17E-02
ENSG00000185046	56899	ANKS1B	1.01	2.16E-04	4.82E-02
ENSG00000165949	3429	IFI27	-1.05	2.14E-04	4.82E-02
ENSG00000186847	3861	KRT14	1.20	2.30E-04	5.04E-02
ENSG00000136929	55363	HEMGN	1.19	2.35E-04	5.04E-02
ENSG00000167107	80221	ACSF2	-0.55	2.35E-04	5.04E-02
ENSG00000169129	84632	AFAP1L2	0.87	2.45E-04	5.19E-02
ENSG00000134762	1825	DSC3	1.10	2.86E-04	5.88E-02
ENSG00000212743	NA		0.96	2.85E-04	5.88E-02
ENSG00000149575	6327	SCN2B	1.13	3.00E-04	6.10E-02
ENSG00000188257	5320	PLA2G2A	1.16	3.22E-04	6.15E-02
ENSG00000102935	23090	ZNF423	1.02	3.15E-04	6.15E-02
ENSG00000143320	1382	CRABP2	0.99	3.22E-04	6.15E-02
ENSG00000138660	55435	AP1AR	-0.49	3.07E-04	6.15E-02
ENSG00000157601	4599	MX1	-0.88	3.15E-04	6.15E-02
ENSG00000171847	55138	FAM90A1	0.80	3.36E-04	6.22E-02
ENSG00000115461	3488	IGFBP5	0.71	3.37E-04	6.22E-02
ENSG00000151632	1646	AKR1C2	-1.17	3.36E-04	6.22E-02
ENSG00000189306	27341	RRP7A	0.44	3.42E-04	6.24E-02
ENSG00000148541	220965	FAM13C	0.72	3.60E-04	6.49E-02
ENSG00000111725	5564	PRKAB1	-0.49	3.65E-04	6.50E-02
ENSG00000182885	222487	ADGRG3	1.15	3.74E-04	6.54E-02
ENSG00000206172	3039	HBA1	1.15	3.80E-04	6.54E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000157502	139221	MUM1L1	1.09	3.77E-04	6.54E-02
ENSG00000169891	9185	REPS2	-0.46	3.88E-04	6.54E-02
ENSG00000129437	43847	KLK14	-1.09	3.85E-04	6.54E-02
ENSG00000211956	NA	IGHV4-34	1.15	3.93E-04	6.57E-02
ENSG00000106483	6424	SFRP4	1.15	4.19E-04	6.75E-02
ENSG00000175879	3234	HOXD8	1.13	4.36E-04	6.75E-02
ENSG00000166947	2038	EPB42	1.04	4.35E-04	6.75E-02
ENSG00000028310	65980	BRD9	0.17	4.15E-04	6.75E-02
ENSG00000134046	8932	MBD2	-0.18	4.39E-04	6.75E-02
ENSG00000011007	6924	TCEB3	-0.29	4.10E-04	6.75E-02
ENSG00000198722	10497	UNC13B	-0.46	4.24E-04	6.75E-02
ENSG00000182795	79098	C1orf116	-0.80	4.33E-04	6.75E-02
ENSG00000110042	23220	DTX4	-0.40	4.44E-04	6.78E-02
ENSG00000169612	83640	FAM103A1	-0.67	4.56E-04	6.88E-02
ENSG00000139517	222484	LNX2	-0.38	4.66E-04	6.98E-02
ENSG00000165966	29951	PDZRN4	1.14	4.80E-04	7.01E-02
ENSG00000196109	163223	ZNF676	1.12	4.81E-04	7.01E-02
ENSG00000188517	84570	COL25A1	1.12	4.86E-04	7.01E-02
ENSG00000121898	119587	CPXM2	1.07	4.83E-04	7.01E-02
ENSG00000185739	6345	SRL	1.13	5.01E-04	7.10E-02
ENSG00000166033	5654	HTRA1	0.82	4.98E-04	7.10E-02
ENSG00000179772	2307	FOXS1	1.13	5.20E-04	7.17E-02
ENSG00000172137	794	CALB2	1.13	5.19E-04	7.17E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000086544	80271	ITPKC	-0.42	5.15E-04	7.17E-02
ENSG00000130513	9518	GDF15	-1.04	5.29E-04	7.17E-02
ENSG00000129451	5655	KLK10	-1.05	5.26E-04	7.17E-02
ENSG00000109472	1363	CPE	1.03	5.40E-04	7.20E-02
ENSG00000171206	81603	TRIM8	-0.30	5.38E-04	7.20E-02
ENSG00000128285	2847	MCHR1	1.09	5.60E-04	7.40E-02
ENSG00000197993	3792	KEL	1.08	5.68E-04	7.45E-02
ENSG00000138642	55008	HERC6	-0.82	5.78E-04	7.51E-02
ENSG00000162729	93185	IGSF8	0.32	5.93E-04	7.65E-02
ENSG00000105369	973	CD79A	1.11	6.54E-04	7.67E-02
ENSG00000146374	84870	RSPO3	1.11	6.56E-04	7.67E-02
ENSG00000167483	199786	FAM129C	1.11	6.75E-04	7.67E-02
ENSG00000205038	93035	PKHD1L1	1.07	6.67E-04	7.67E-02
ENSG00000158560	1780	DYNC1I1	1.07	6.63E-04	7.67E-02
ENSG00000101000	10544	PROCR	1.04	6.65E-04	7.67E-02
ENSG00000197410	54798	DCHS2	1.03	6.66E-04	7.67E-02
ENSG00000137573	23213	SULF1	1.02	6.74E-04	7.67E-02
ENSG00000091972	4345	CD200	1.01	6.81E-04	7.67E-02
ENSG00000161381	57125	PLXDC1	0.97	6.23E-04	7.67E-02
ENSG00000067840	57595	PDZD4	0.94	6.64E-04	7.67E-02
ENSG00000244363	NA	RPL7P23	0.77	6.71E-04	7.67E-02
ENSG00000141698	115024	NT5C3B	0.41	6.51E-04	7.67E-02
ENSG00000184056	26276	VPS33B	0.24	6.73E-04	7.67E-02
ENSG00000226742	440498	HSBP1L1	-0.37	6.66E-04	7.67E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000064666	1265	CNN2	-0.38	6.73E-04	7.67E-02
ENSG00000198142	65124	SOWAHC	-0.53	6.36E-04	7.67E-02
ENSG00000198183	51297	BPIFA1	-1.09	6.79E-04	7.67E-02
ENSG00000185271	123103	KLHL33	1.10	7.36E-04	7.96E-02
ENSG00000143248	8490	RGS5	1.10	7.26E-04	7.96E-02
ENSG00000106018	7434	VIPR2	1.10	7.45E-04	7.96E-02
ENSG00000168542	1281	COL3A1	1.10	7.33E-04	7.96E-02
ENSG00000186105	100130733	LRRC70	0.93	7.44E-04	7.96E-02
ENSG00000265150	NA	NA	0.87	7.23E-04	7.96E-02
ENSG00000172840	57546	PDP2	-0.34	7.47E-04	7.96E-02
ENSG00000156675	80223	RAB11FIP1	-0.61	7.27E-04	7.96E-02
ENSG00000108001	253738	EBF3	1.10	7.84E-04	8.11E-02
ENSG00000114948	8745	ADAM23	1.08	7.82E-04	8.11E-02
ENSG00000106404	24146	CLDN15	1.08	7.73E-04	8.11E-02
ENSG00000139910	4857	NOVA1	1.07	7.92E-04	8.11E-02
ENSG00000146802	64418	TMEM168	0.33	7.92E-04	8.11E-02
ENSG00000183486	4600	MX2	-0.91	7.89E-04	8.11E-02
ENSG00000157766	176	ACAN	1.08	8.06E-04	8.16E-02
ENSG00000214174	201283	AMZ2P1	-0.54	8.08E-04	8.16E-02
ENSG00000185305	54622	ARL15	0.67	8.21E-04	8.19E-02
ENSG00000137709	25833	POU2F3	-0.67	8.17E-04	8.19E-02
ENSG00000117707	5629	PROX1	0.99	8.39E-04	8.31E-02
ENSG00000144619	152330	CNTN4	0.77	8.54E-04	8.41E-02
ENSG00000158578	212	ALAS2	1.08	8.94E-04	8.58E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000228570	NA	NUTM2E	1.08	9.23E-04	8.58E-02
ENSG00000163735	6374	CXCL5	1.08	9.30E-04	8.58E-02
ENSG00000167476	126306	JSRP1	1.07	9.11E-04	8.58E-02
ENSG00000116833	2494	NR5A2	1.06	8.81E-04	8.58E-02
ENSG00000120322	56128	PCDHB8	1.05	9.31E-04	8.58E-02
ENSG00000238741	677767	SCARNA7	0.37	9.26E-04	8.58E-02
ENSG00000169093	8623	ASMTL	0.23	9.12E-04	8.58E-02
ENSG00000081026	260425	MAGI3	-0.61	9.06E-04	8.58E-02
ENSG00000103528	51760	SYT17	-0.62	9.00E-04	8.58E-02
ENSG00000100033	5625	PRODH	-0.81	9.09E-04	8.58E-02
ENSG00000165868	259217	HSPA12A	0.86	9.54E-04	8.75E-02
ENSG00000163251	7855	FZD5	-0.74	9.87E-04	9.00E-02
ENSG00000122140	51116	MRPS2	-0.38	9.93E-04	9.00E-02
ENSG00000171792	83695	RHNO1	-0.48	1.00E-03	9.02E-02
ENSG00000104369	56704	JPH1	-0.58	1.03E-03	9.20E-02
ENSG00000168079	286133	SCARA5	1.07	1.03E-03	9.21E-02
ENSG00000092295	7051	TGM1	1.00	1.04E-03	9.22E-02
ENSG00000106809	4969	OGN	1.07	1.06E-03	9.35E-02
ENSG00000163534	115350	FCRL1	1.06	1.11E-03	9.44E-02
ENSG00000091137	5172	SLC26A4	1.00	1.10E-03	9.44E-02
ENSG00000099958	91319	DERL3	0.89	1.15E-03	9.44E-02
ENSG00000153253	6328	SCN3A	0.82	1.16E-03	9.44E-02
ENSG00000251402	NA	FAM90A25P	0.74	1.12E-03	9.44E-02

Table 9. All samples Ensembl ID	Entrez ID	Gene symbol	log2 Fold Change (UIP/NonUIP)	p value	FDR p value
ENSG00000120327	56122	PCDHB14	0.72	1.09E-03	9.44E-02
ENSG00000138795	51176	LEF1	0.64	1.13E-03	9.44E-02
ENSG00000144040	94097	SFXN5	0.44	1.12E-03	9.44E-02
ENSG00000155463	5018	OXA1L	-0.20	1.15E-03	9.44E-02
ENSG00000136830	64855	FAM129B	-0.24	1.15E-03	9.44E-02
ENSG00000141452	29919	C18orf8	-0.30	1.15E-03	9.44E-02
ENSG00000148730	1979	EIF4EBP2	-0.33	1.12E-03	9.44E-02
ENSG00000115415	6772	STAT1	-0.86	1.10E-03	9.44E-02
ENSG00000126709	2537	IFI6	-1.03	1.15E-03	9.44E-02
ENSG00000095951	3096	HIVEP1	-0.24	1.20E-03	9.76E-02
ENSG00000187942	401944	LDLRAD2	0.97	1.21E-03	9.80E-02
ENSG00000105928	1687	DFNA5	0.83	1.22E-03	9.81E-02
ENSG00000224397	NA	LINC01272	1.02	1.23E-03	9.83E-02
ENSG00000170011	25924	MYRIP	0.86	1.32E-03	1.00E-01
ENSG00000120784	22835	ZFP30	0.37	1.30E-03	1.00E-01
ENSG00000170185	84640	USP38	-0.26	1.31E-03	1.00E-01
ENSG00000076513	88455	ANKRD13A	-0.31	1.26E-03	1.00E-01
ENSG00000135148	10906	TRAFD1	-0.32	1.28E-03	1.00E-01
ENSG00000170085	375484	SIMC1	-0.43	1.32E-03	1.00E-01
ENSG00000175324	27257	LSM1	-0.45	1.30E-03	1.00E-01
ENSG00000141738	2886	GRB7	-0.54	1.28E-03	1.00E-01
ENSG00000088002	6820	SULT2B1	-0.75	1.28E-03	1.00E-01
ENSG00000010030	51513	ETV7	-0.86	1.29E-03	1.00E-01
ENSG00000137959	10964	IFI44L	-1.01	1.27E-03	1.00E-01

UIP (n=9 samples); Non UIP (n=15 samples). Positive log2 fold change value indicates over-expression in UIP relative to Non UIP; negative log2 value indicates under-expression in UIP relative to Non UIP. In this analysis the smoking history status of the patients involved was not evaluated, and the cohort harbored both smokers and non-smokers.

[0185] Table 10. Differentially expressed genes in non-smoker UIP vs. non-smoker Non-UIP samples.

Table 10. Non-smokers **Ensembl ID**	**Entrez ID**	**Gene symbol**	**Log2 Fold Change (UIP/NonUIP)**	**P value**	**FDR P value**
ENSG00000133687	83857	TMTC1	1.72	4.52E-21	6.68E-17
ENSG00000169129	84632	AFAP1L2	1.63	1.62E-19	1.19E-15
ENSG00000180229	283755	HERC2P3	2.52	9.77E-15	4.81E-11
ENSG00000107518	26033	ATRNL1	3.00	2.49E-14	9.21E-11
ENSG00000198380	2673	GFPT1	-0.62	9.55E-14	2.35E-10
ENSG00000211976	NA	IGHV3-73	2.21	9.47E-14	2.35E-10
ENSG00000114902	28972	SPCS1	-0.64	1.99E-12	4.20E-09
ENSG00000108001	253738	EBF3	2.77	3.39E-12	6.27E-09
ENSG00000154122	56172	ANKH	1.02	4.17E-12	6.85E-09
ENSG00000012660	60481	ELOVL5	-0.66	6.94E-12	1.03E-08
ENSG00000119888	4072	EPCAM	-1.04	8.67E-12	1.16E-08
ENSG00000147676	114569	MAL2	-1.08	1.83E-11	1.94E-08
ENSG00000148357	256158	HMCN2	1.99	1.62E-11	1.94E-08
ENSG00000185499	4582	MUC1	-1.09	1.84E-11	1.94E-08
ENSG00000157557	2114	ETS2	0.73	8.18E-11	8.06E-08
ENSG00000151789	79750	ZNF385D	2.73	1.04E-10	9.62E-08
ENSG00000213088	2532	ACKR1	2.18	3.53E-10	3.06E-07
ENSG00000038210	55300	PI4K2B	-0.77	4.14E-10	3.40E-07
ENSG00000105426	5802	PTPRS	0.80	9.94E-10	7.73E-07
ENSG00000100034	9647	PPM1F	1.27	1.17E-09	8.14E-07

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000112562	64094	SMOC2	1.70	1.21E-09	8.14E-07
ENSG00000139211	347902	AMIGO2	0.93	1.15E-09	8.14E-07
ENSG00000130158	57572	DOCK6	0.75	1.42E-09	9.10E-07
ENSG00000124145	6385	SDC4	-0.67	2.44E-09	1.44E-06
ENSG00000129255	9526	MPDU1	-0.54	2.44E-09	1.44E-06
ENSG00000100219	7494	XBP1	-1.02	2.76E-09	1.48E-06
ENSG00000120318	64411	ARAP3	0.93	2.80E-09	1.48E-06
ENSG00000144136	6574	SLC20A1	-0.65	2.63E-09	1.48E-06
ENSG00000140873	170692	ADAMTS18	2.27	3.32E-09	1.69E-06
ENSG00000120693	4093	SMAD9	1.43	4.04E-09	1.99E-06
ENSG00000080007	55510	DDX43	-2.11	6.55E-09	3.12E-06
ENSG00000105737	2901	GRIK5	2.34	8.09E-09	3.54E-06
ENSG00000157064	23057	NMNAT2	2.14	8.14E-09	3.54E-06
ENSG00000161381	57125	PLXDC1	1.21	7.73E-09	3.54E-06
ENSG00000154736	11096	ADAMTS5	1.92	1.01E-08	4.13E-06
ENSG00000185046	56899	ANKS1B	1.51	9.78E-09	4.13E-06
ENSG00000104213	5157	PDGFRL	2.09	1.19E-08	4.52E-06
ENSG00000166398	9710	KIAA0355	0.82	1.19E-08	4.52E-06
ENSG00000189058	347	APOD	1.55	1.14E-08	4.52E-06
ENSG00000102935	23090	ZNF423	1.47	1.34E-08	4.86E-06
ENSG00000111145	2004	ELK3	0.89	1.35E-08	4.86E-06
ENSG00000138160	3832	KIF11	-1.35	1.44E-08	5.06E-06
ENSG00000197147	23507	LRRC8B	-0.89	1.80E-08	6.19E-06
ENSG00000131386	117248	GALNT15	2.41	1.95E-08	6.39E-06

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000177839	56127	PCDHB9	1.87	1.91E-08	6.39E-06
ENSG00000114446	55081	IFT57	-0.65	2.20E-08	7.06E-06
ENSG00000198932	9737	GPRASP1	0.90	2.34E-08	7.36E-06
ENSG00000178031	92949	ADAMTSL1	2.30	2.50E-08	7.70E-06
ENSG00000230989	3281	HSBP1	-0.74	2.59E-08	7.80E-06
ENSG00000100065	29775	CARD10	1.69	2.91E-08	8.60E-06
ENSG00000142798	3339	HSPG2	1.20	3.13E-08	9.06E-06
ENSG00000134533	85004	RERG	1.68	3.70E-08	1.05E-05
ENSG00000105784	154661	RUNDC3B	2.28	3.87E-08	1.08E-05
ENSG00000039068	999	CDH1	-0.87	6.14E-08	1.55E-05
ENSG00000104549	6713	SQLE	-1.38	6.10E-08	1.55E-05
ENSG00000105996	3199	HOXA2	1.60	5.72E-08	1.55E-05
ENSG00000130234	59272	ACE2	-1.52	6.19E-08	1.55E-05
ENSG00000162881	165140	OXER1	1.30	5.81E-08	1.55E-05
ENSG00000177098	6330	SCN4B	1.12	6.10E-08	1.55E-05
ENSG00000007312	974	CD79B	2.13	6.62E-08	1.58E-05
ENSG00000163898	200879	LIPH	-0.91	6.52E-08	1.58E-05
ENSG00000189377	284340	CXCL17	-1.17	6.53E-08	1.58E-05
ENSG00000198814	2710	GK	-0.83	6.79E-08	1.59E-05
ENSG00000076944	6813	STXBP2	-0.56	7.52E-08	1.71E-05
ENSG00000153902	163175	LGI4	1.74	7.53E-08	1.71E-05
ENSG00000117448	10327	AKR1A1	-0.63	7.74E-08	1.73E-05
ENSG00000164530	221476	PI16	2.17	8.33E-08	1.84E-05
ENSG00000233297	NA	RASA4DP	2.29	9.09E-08	1.97E-05

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000182718	302	ANXA2	-0.62	9.95E-08	2.13E-05
ENSG00000110455	84680	ACCS	0.98	1.03E-07	2.17E-05
ENSG00000197253	64499	TPSB2	2.07	1.10E-07	2.29E-05
ENSG00000125999	92747	BPIFB1	-1.76	1.15E-07	2.37E-05
ENSG00000211936	NA	NA	2.23	1.22E-07	2.47E-05
ENSG00000108852	4355	MPP2	1.68	1.28E-07	2.55E-05
ENSG00000119514	79695	GALNT12	-0.60	1.64E-07	3.18E-05
ENSG00000146021	26249	KLHL3	1.36	1.63E-07	3.18E-05
ENSG00000173702	56667	MUC13	-2.33	1.78E-07	3.41E-05
ENSG00000116748	270	AMPD1	1.80	1.85E-07	3.50E-05
ENSG00000196411	2050	EPHB4	0.86	2.09E-07	3.91E-05
ENSG00000086061	3301	DNAJA1	-0.73	2.20E-07	4.07E-05
ENSG00000074181	4854	NOTCH3	0.92	2.24E-07	4.07E-05
ENSG00000181234	92293	TMEM132C	2.08	2.26E-07	4.07E-05
ENSG00000074590	9891	NUAK1	1.27	2.47E-07	4.39E-05
ENSG00000154263	10349	ABCA10	1.44	2.92E-07	5.14E-05
ENSG00000186322	NA	NA	1.99	3.30E-07	5.74E-05
ENSG00000130164	3949	LDLR	-1.00	3.36E-07	5.77E-05
ENSG00000133935	11161	C14orf1	-0.76	3.54E-07	5.99E-05
ENSG00000151892	2674	GFRA1	1.96	3.57E-07	5.99E-05
ENSG00000196628	6925	TCF4	0.93	3.65E-07	6.06E-05
ENSG00000106070	2887	GRB10	1.22	3.94E-07	6.46E-05
ENSG00000076555	32	ACACB	1.06	4.48E-07	7.27E-05
ENSG00000173947	128344	PIFO	-1.26	4.74E-07	7.61E-05

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000004468	952	CD38	-1.40	5.09E-07	8.08E-05
ENSG00000154330	5239	PGM5	1.72	5.62E-07	8.83E-05
ENSG00000085185	63035	BCORL1	0.61	5.71E-07	8.88E-05
ENSG00000157933	6497	SKI	0.57	5.77E-07	8.89E-05
ENSG00000080854	22997	IGSF9B	1.56	6.01E-07	9.16E-05
ENSG00000244734	3043	HBB	2.28	6.25E-07	9.42E-05
ENSG00000080572	139212	PIH1D3	-1.44	6.80E-07	1.02E-04
ENSG00000154529	728577	CNTNAP3B	1.83	7.23E-07	1.07E-04
ENSG00000142731	10733	PLK4	-1.30	9.19E-07	1.34E-04
ENSG00000146425	6993	DYNLT1	-1.05	9.34E-07	1.35E-04
ENSG00000109861	1075	CTSC	-0.92	9.89E-07	1.42E-04
ENSG00000118640	8673	VAMP8	-0.83	1.00E-06	1.43E-04
ENSG00000091262	368	ABCC6	-0.97	1.05E-06	1.48E-04
ENSG00000012124	933	CD22	1.94	1.08E-06	1.48E-04
ENSG00000079337	10411	RAPGEF3	1.63	1.09E-06	1.48E-04
ENSG00000113161	3156	HMGCR	-1.13	1.09E-06	1.48E-04
ENSG00000183346	219621	C10orf107	-1.22	1.07E-06	1.48E-04
ENSG00000197705	57565	KLHL14	2.01	1.11E-06	1.49E-04
ENSG00000215217	134121	C5orf49	-1.20	1.14E-06	1.52E-04
ENSG00000070190	27071	DAPP1	-1.19	1.19E-06	1.55E-04
ENSG00000165434	283209	PGM2L1	-0.92	1.18E-06	1.55E-04
ENSG00000102738	10240	MRPS31	-0.97	1.22E-06	1.56E-04
ENSG00000132698	57111	RAB25	-0.79	1.22E-06	1.56E-04
ENSG00000186105	100130733	LRRC70	1.77	1.24E-06	1.58E-04

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000123983	2181	ACSL3	-0.87	1.38E-06	1.72E-04
ENSG00000188010	729967	MORN2	-1.27	1.37E-06	1.72E-04
ENSG00000138670	153020	RASGEF1B	-0.83	1.56E-06	1.94E-04
ENSG00000152104	5784	PTPN14	0.94	1.62E-06	1.98E-04
ENSG00000165995	783	CACNB2	1.24	1.62E-06	1.98E-04
ENSG00000109586	51809	GALNT7	-0.94	1.73E-06	2.10E-04
ENSG00000129946	25759	SHC2	1.66	1.82E-06	2.18E-04
ENSG00000139910	4857	NOVA1	2.15	1.99E-06	2.37E-04
ENSG00000188175	253012	HEPACAM2	-1.75	2.01E-06	2.37E-04
ENSG00000174405	3981	LIG4	-0.58	2.04E-06	2.37E-04
ENSG00000181885	1366	CLDN7	-1.13	2.03E-06	2.37E-04
ENSG00000187134	1645	AKR1C1	-0.86	2.07E-06	2.39E-04
ENSG00000253731	56109	PCDHGA6	1.19	2.09E-06	2.39E-04
ENSG00000090006	8425	LTBP4	1.38	2.13E-06	2.42E-04
ENSG00000176438	161176	SYNE3	0.98	2.18E-06	2.44E-04
ENSG00000188536	3040	HBA2	2.17	2.17E-06	2.44E-04
ENSG00000141198	10040	TOM1L1	-0.57	2.27E-06	2.50E-04
ENSG00000164124	55314	TMEM144	-0.88	2.26E-06	2.50E-04
ENSG00000068912	27248	ERLEC1	-0.48	2.33E-06	2.55E-04
ENSG00000167779	3489	IGFBP6	1.85	2.44E-06	2.65E-04
ENSG00000159212	54102	CLIC6	-0.90	2.50E-06	2.70E-04
ENSG00000116833	2494	NR5A2	1.94	2.54E-06	2.72E-04
ENSG00000197614	8076	MFAP5	2.15	2.70E-06	2.87E-04
ENSG00000152518	678	ZFP36L2	0.79	2.81E-06	2.96E-04

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000177156	6888	TALDO1	-0.45	2.83E-06	2.96E-04
ENSG00000144619	152330	CNTN4	0.79	2.89E-06	3.01E-04
ENSG00000124772	57699	CPNE5	1.80	2.99E-06	3.04E-04
ENSG00000166908	79837	PIP4K2C	-0.49	2.99E-06	3.04E-04
ENSG00000168765	2948	GSTM4	0.72	2.95E-06	3.04E-04
ENSG00000105928	1687	DFNA5	1.47	3.02E-06	3.05E-04
ENSG00000186471	158798	AKAP14	-1.21	3.04E-06	3.05E-04
ENSG00000183604	NA	SMG1P5	1.68	3.12E-06	3.11E-04
ENSG00000182272	338707	B4GALNT4	1.65	3.32E-06	3.29E-04
ENSG00000170017	214	ALCAM	-1.08	3.37E-06	3.32E-04
ENSG00000163191	6282	S100A11	-0.92	3.77E-06	3.69E-04
ENSG00000100243	1727	CYB5R3	0.31	3.83E-06	3.70E-04
ENSG00000119616	51077	FCF1	-0.57	3.81E-06	3.70E-04
ENSG00000106852	26468	LHX6	1.71	3.86E-06	3.70E-04
ENSG00000164056	10252	SPRY1	1.10	4.26E-06	4.06E-04
ENSG00000256870	160728	SLC5A8	-1.68	4.31E-06	4.08E-04
ENSG00000112110	29074	MRPL18	-0.74	4.36E-06	4.10E-04
ENSG00000243716	440345	NPIPB5	0.46	4.42E-06	4.13E-04
ENSG00000182093	7485	WRB	-0.58	4.63E-06	4.30E-04
ENSG00000213398	3931	LCAT	0.87	4.66E-06	4.31E-04
ENSG00000079459	2222	FDFT1	-0.89	4.75E-06	4.36E-04
ENSG00000163082	130367	SGPP2	-0.75	4.82E-06	4.40E-04
ENSG00000181722	26137	ZBTB20	0.71	4.96E-06	4.50E-04
ENSG00000152953	55351	STK32B	2.03	5.05E-06	4.55E-04

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000070193	2255	FGF10	1.89	5.24E-06	4.67E-04
ENSG00000106404	24146	CLDN15	2.03	5.27E-06	4.67E-04
ENSG00000107281	56654	NPDC1	1.21	5.22E-06	4.67E-04
ENSG00000107949	56647	BCCIP	-0.61	5.32E-06	4.68E-04
ENSG00000161055	92304	SCGB3A1	-1.74	6.01E-06	5.25E-04
ENSG00000087916	NA	NA	-1.75	6.14E-06	5.34E-04
ENSG00000119139	9414	TJP2	-0.47	6.21E-06	5.36E-04
ENSG00000151468	83643	CCDC3	1.74	6.27E-06	5.39E-04
ENSG00000205809	3822	KLRC2	-1.67	6.68E-06	5.70E-04
ENSG00000131374	9779	TBC1D5	0.35	6.76E-06	5.71E-04
ENSG00000178741	9377	COX5A	-0.63	6.73E-06	5.71E-04
ENSG00000075142	6717	SRI	-0.78	6.86E-06	5.73E-04
ENSG00000141720	NA	NA	0.40	6.87E-06	5.73E-04
ENSG00000159167	6781	STC1	1.91	6.99E-06	5.80E-04
ENSG00000081818	56131	PCDHB4	1.46	7.29E-06	5.95E-04
ENSG00000135773	10753	CAPN9	-0.90	7.27E-06	5.95E-04
ENSG00000165300	26050	SLITRK5	1.88	7.26E-06	5.95E-04
ENSG00000133321	5920	RARRES3	-1.58	7.92E-06	6.43E-04
ENSG00000160447	29941	PKN3	1.30	8.24E-06	6.66E-04
ENSG00000136859	23452	ANGPTL2	1.38	8.33E-06	6.69E-04
ENSG00000222009	149478	BTBD19	1.14	8.54E-06	6.82E-04
ENSG00000185250	285755	PPIL6	-1.17	8.59E-06	6.83E-04
ENSG00000164330	1879	EBF1	1.73	8.71E-06	6.86E-04
ENSG00000182771	2894	GRID1	1.81	8.73E-06	6.86E-04

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000137842	80021	TMEM62	-0.91	8.80E-06	6.88E-04
ENSG00000088538	1795	DOCK3	1.25	8.88E-06	6.91E-04
ENSG00000178966	80010	RMI1	-0.93	9.01E-06	6.97E-04
ENSG00000133665	84332	DYDC2	-1.18	9.18E-06	7.06E-04
ENSG00000117322	1380	CR2	2.00	9.44E-06	7.23E-04
ENSG00000100170	6523	SLC5A1	-1.54	1.00E-05	7.61E-04
ENSG00000101384	182	JAG1	0.73	1.00E-05	7.61E-04
ENSG00000120756	5357	PLS1	-1.01	1.10E-05	8.29E-04
ENSG00000102098	10389	SCML2	1.41	1.12E-05	8.41E-04
ENSG00000114200	590	BCHE	1.93	1.13E-05	8.41E-04
ENSG00000013275	5704	PSMC4	-0.67	1.15E-05	8.50E-04
ENSG00000108953	7531	YWHAE	-0.48	1.15E-05	8.50E-04
ENSG00000140181	NA	NA	1.02	1.17E-05	8.53E-04
ENSG00000152939	153562	MARVELD2	-0.86	1.16E-05	8.53E-04
ENSG00000162882	23498	HAAO	1.22	1.28E-05	9.29E-04
ENSG00000088448	55608	ANKRD10	0.59	1.31E-05	9.49E-04
ENSG00000272636	8447	DOC2B	1.36	1.34E-05	9.64E-04
ENSG00000052802	6307	MSMO1	-1.29	1.36E-05	9.70E-04
ENSG00000077063	83992	CTTNBP2	1.25	1.37E-05	9.70E-04
ENSG00000112972	3157	HMGCS1	-1.11	1.36E-05	9.70E-04
ENSG00000133019	1131	CHRM3	1.44	1.37E-05	9.70E-04
ENSG00000069329	55737	VPS35	-0.48	1.40E-05	9.88E-04
ENSG00000166265	116159	CYYR1	1.63	1.42E-05	9.94E-04
ENSG00000159399	3099	HK2	-1.04	1.44E-05	1.00E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000257057	NA	C11orf97	-1.40	1.47E-05	1.02E-03
ENSG00000065618	1308	COL17A1	1.62	1.52E-05	1.05E-03
ENSG00000163624	1040	CDS1	-1.01	1.67E-05	1.14E-03
ENSG00000164764	157869	SBSPON	1.82	1.67E-05	1.14E-03
ENSG00000171444	4163	MCC	0.99	1.67E-05	1.14E-03
ENSG00000183323	202243	CCDC125	-1.05	1.66E-05	1.14E-03
ENSG00000152332	127933	UHMK1	-0.55	1.71E-05	1.16E-03
ENSG00000205277	10071	MUC12	1.47	1.75E-05	1.18E-03
ENSG00000091592	22861	NLRP1	0.94	1.77E-05	1.18E-03
ENSG00000101230	140862	ISM1	1.31	1.77E-05	1.18E-03
ENSG00000112981	8382	NME5	-1.10	1.79E-05	1.18E-03
ENSG00000196263	57573	ZNF471	0.81	1.79E-05	1.18E-03
ENSG00000149809	7108	TM7SF2	-0.74	1.81E-05	1.19E-03
ENSG00000139644	7009	TMBIM6	-0.60	1.83E-05	1.20E-03
ENSG00000116138	23341	DNAJC16	-0.51	1.88E-05	1.21E-03
ENSG00000143248	8490	RGS5	1.70	1.88E-05	1.21E-03
ENSG00000183644	399949	C11orf88	-1.23	1.88E-05	1.21E-03
ENSG00000109814	7358	UGDH	-0.76	1.89E-05	1.22E-03
ENSG00000131475	84313	VPS25	-0.57	1.94E-05	1.24E-03
ENSG00000183454	2903	GRIN2A	1.94	1.98E-05	1.26E-03
ENSG00000057252	6646	SOAT1	-0.77	2.07E-05	1.31E-03
ENSG00000106123	2051	EPHB6	1.12	2.11E-05	1.31E-03
ENSG00000133067	59352	LGR6	1.51	2.11E-05	1.31E-03
ENSG00000138356	316	AOX1	1.79	2.09E-05	1.31E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000173269	79812	MMRN2	1.69	2.09E-05	1.31E-03
ENSG00000186642	5138	PDE2A	1.94	2.12E-05	1.31E-03
ENSG00000178053	4291	MLF1	-1.06	2.15E-05	1.33E-03
ENSG00000087995	339175	METTL2A	-0.57	2.18E-05	1.34E-03
ENSG00000130600	283120	H19	1.87	2.18E-05	1.34E-03
ENSG00000114573	523	ATP6V1A	-0.62	2.24E-05	1.35E-03
ENSG00000131203	3620	IDO1	-1.91	2.23E-05	1.35E-03
ENSG00000143036	126969	SLC44A3	-0.74	2.21E-05	1.35E-03
ENSG00000262209	56102	PCDHGB3	1.37	2.22E-05	1.35E-03
ENSG00000168079	286133	SCARA5	1.77	2.29E-05	1.37E-03
ENSG00000163534	115350	FCRL1	1.93	2.35E-05	1.40E-03
ENSG00000165304	9833	MELK	-1.58	2.36E-05	1.40E-03
ENSG00000101421	128866	CHMP4B	-0.48	2.37E-05	1.41E-03
ENSG00000162961	84661	DPY30	-0.75	2.53E-05	1.49E-03
ENSG00000188931	257177	CFAP126	-1.15	2.53E-05	1.49E-03
ENSG00000129467	196883	ADCY4	1.60	2.55E-05	1.49E-03
ENSG00000173530	8793	TNFRSF10D	0.83	2.54E-05	1.49E-03
ENSG00000158683	168507	PKD1L1	1.68	2.68E-05	1.56E-03
ENSG00000000003	7105	TSPAN6	-1.01	2.70E-05	1.56E-03
ENSG00000135679	4193	MDM2	-0.59	2.70E-05	1.56E-03
ENSG00000241244	NA	IGKV1D-16	1.79	2.76E-05	1.58E-03
ENSG00000086548	4680	CEACAM6	-1.11	2.86E-05	1.64E-03
ENSG00000005108	221981	THSD7A	1.06	2.87E-05	1.64E-03
ENSG00000163001	112942	CFAP36	-0.57	2.91E-05	1.65E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000178445	2731	GLDC	1.32	2.92E-05	1.65E-03
ENSG00000196586	4646	MYO6	-0.56	2.94E-05	1.66E-03
ENSG00000130300	83483	PLVAP	1.89	2.97E-05	1.66E-03
ENSG00000173068	54796	BNC2	1.44	2.98E-05	1.66E-03
ENSG00000211972	NA	IGHV3-66	1.82	2.98E-05	1.66E-03
ENSG00000163531	23114	NFASC	0.75	3.10E-05	1.72E-03
ENSG00000145287	51316	PLAC8	-1.30	3.13E-05	1.72E-03
ENSG00000157764	673	BRAF	0.36	3.14E-05	1.72E-03
ENSG00000197959	26052	DNM3	1.09	3.12E-05	1.72E-03
ENSG00000119138	687	KLF9	1.20	3.18E-05	1.74E-03
ENSG00000185760	56479	KCNQ5	1.62	3.26E-05	1.78E-03
ENSG00000066382	744	MPPED2	1.28	3.35E-05	1.80E-03
ENSG00000088986	8655	DYNLL1	-0.77	3.32E-05	1.80E-03
ENSG00000104413	54845	ESRP1	-0.77	3.35E-05	1.80E-03
ENSG00000116906	8443	GNPAT	-0.33	3.32E-05	1.80E-03
ENSG00000165716	138311	FAM69B	1.58	3.34E-05	1.80E-03
ENSG00000163263	388701	C1orf189	-1.27	3.44E-05	1.83E-03
ENSG00000163993	6286	S100P	-1.73	3.46E-05	1.84E-03
ENSG00000102287	2564	GABRE	1.02	3.59E-05	1.90E-03
ENSG00000011523	23177	CEP68	0.74	3.67E-05	1.94E-03
ENSG00000149212	143686	SESN3	0.83	3.74E-05	1.96E-03
ENSG00000174059	947	CD34	1.78	3.73E-05	1.96E-03
ENSG00000178125	286187	PPP1R42	-1.05	3.74E-05	1.96E-03
ENSG00000145494	4726	NDUFS6	-0.39	3.84E-05	2.00E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000166562	90701	SEC11C	-0.65	3.97E-05	2.06E-03
ENSG00000261934	56107	PCDHGA9	0.90	3.99E-05	2.06E-03
ENSG00000126895	554	AVPR2	1.24	4.01E-05	2.06E-03
ENSG00000131844	64087	MCCC2	-0.44	4.04E-05	2.07E-03
ENSG00000166813	374654	KIF7	1.34	4.12E-05	2.11E-03
ENSG00000185245	2811	GP1BA	1.18	4.22E-05	2.15E-03
ENSG00000057704	57458	TMCC3	1.54	4.29E-05	2.18E-03
ENSG00000148541	220965	FAM13C	1.17	4.38E-05	2.21E-03
ENSG00000224114	NA		-1.66	4.44E-05	2.24E-03
ENSG00000068796	3796	KIF2A	-0.75	4.51E-05	2.26E-03
ENSG00000254986	10072	DPP3	-0.57	4.52E-05	2.26E-03
ENSG00000266714	NA	MYO15B	0.93	4.52E-05	2.26E-03
ENSG00000145824	9547	CXCL14	1.80	4.67E-05	2.32E-03
ENSG00000262576	56111	PCDHGA4	1.35	4.70E-05	2.33E-03
ENSG00000204604	90333	ZNF468	-0.74	4.96E-05	2.45E-03
ENSG00000111834	345895	RSPH4A	-1.07	5.07E-05	2.46E-03
ENSG00000134202	2947	GSTM3	1.58	4.99E-05	2.46E-03
ENSG00000139193	939	CD27	1.61	5.06E-05	2.46E-03
ENSG00000142687	79932	KIAA0319L	-0.66	5.07E-05	2.46E-03
ENSG00000143653	51097	SCCPDH	-0.61	5.05E-05	2.46E-03
ENSG00000187244	4059	BCAM	0.98	5.00E-05	2.46E-03
ENSG00000225698	NA	IGHV3-72	1.84	5.26E-05	2.54E-03
ENSG00000162896	5284	PIGR	-1.10	5.29E-05	2.54E-03
ENSG00000253485	56110	PCDHGA5	1.40	5.32E-05	2.55E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000103184	9717	SEC14L5	-1.57	5.39E-05	2.57E-03
ENSG00000117399	991	CDC20	-1.23	5.38E-05	2.57E-03
ENSG00000128591	2318	FLNC	1.71	5.43E-05	2.57E-03
ENSG00000153291	9481	SLC25A27	0.80	5.42E-05	2.57E-03
ENSG00000143153	481	ATP1B1	-0.71	5.54E-05	2.62E-03
ENSG00000161798	362	AQP5	-1.15	5.59E-05	2.63E-03
ENSG00000138413	3417	IDH1	-0.56	5.62E-05	2.64E-03
ENSG00000070182	6710	SPTB	1.24	5.67E-05	2.65E-03
ENSG00000165325	159989	CCDC67	-1.20	5.68E-05	2.65E-03
ENSG00000073060	949	SCARB1	1.42	5.92E-05	2.75E-03
ENSG00000120437	39	ACAT2	-1.08	5.99E-05	2.77E-03
ENSG00000181789	22820	COPG1	-0.45	6.01E-05	2.77E-03
ENSG00000265150	NA	NA	1.14	6.00E-05	2.77E-03
ENSG00000211934	NA	IGHV1-2	1.76	6.10E-05	2.80E-03
ENSG00000143772	3707	ITPKB	0.62	6.17E-05	2.82E-03
ENSG00000109846	1410	CRYAB	1.44	6.45E-05	2.93E-03
ENSG00000138031	109	ADCY3	1.04	6.49E-05	2.93E-03
ENSG00000148180	2934	GSN	0.67	6.48E-05	2.93E-03
ENSG00000253910	56103	PCDHGB2	1.36	6.45E-05	2.93E-03
ENSG00000156966	93010	B3GNT7	-0.86	6.54E-05	2.94E-03
ENSG00000130770	93974	ATPIF1	-0.63	6.74E-05	3.02E-03
ENSG00000170312	983	CDK1	-1.01	6.74E-05	3.02E-03
ENSG00000140263	6652	SORD	-1.46	6.79E-05	3.03E-03
ENSG00000139625	7786	MAP3K12	0.86	7.13E-05	3.17E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000089356	5349	FXYD3	-1.11	7.18E-05	3.19E-03
ENSG00000172349	3603	IL16	0.87	7.60E-05	3.36E-03
ENSG00000103534	79838	TMC5	-1.14	7.68E-05	3.39E-03
ENSG00000138175	403	ARL3	-0.75	7.75E-05	3.40E-03
ENSG00000204632	3135	HLA-G	-1.80	7.73E-05	3.40E-03
ENSG00000156675	80223	RAB11FIP1	-0.72	7.88E-05	3.44E-03
ENSG00000081870	51668	HSPB11	-0.85	7.95E-05	3.45E-03
ENSG00000133056	5287	PIK3C2B	0.47	7.94E-05	3.45E-03
ENSG00000133313	55748	CNDP2	-0.77	7.98E-05	3.46E-03
ENSG00000184903	83943	IMMP2L	0.82	8.32E-05	3.60E-03
ENSG00000068976	5837	PYGM	1.50	8.37E-05	3.60E-03
ENSG00000037042	27175	TUBG2	0.71	8.40E-05	3.61E-03
ENSG00000168056	4054	LTBP3	0.85	8.45E-05	3.61E-03
ENSG00000168067	5871	MAP4K2	0.60	8.44E-05	3.61E-03
ENSG00000119630	5228	PGF	1.77	8.51E-05	3.62E-03
ENSG00000116678	3953	LEPR	1.54	8.58E-05	3.64E-03
ENSG00000166226	10576	CCT2	-0.59	8.78E-05	3.72E-03
ENSG00000167088	6632	SNRPD1	-0.54	8.80E-05	3.72E-03
ENSG00000105696	25789	TMEM59L	1.67	8.95E-05	3.73E-03
ENSG00000105711	6324	SCN1B	1.26	8.96E-05	3.73E-03
ENSG00000134339	6289	SAA2	-1.78	8.97E-05	3.73E-03
ENSG00000139631	51380	CSAD	0.90	8.88E-05	3.73E-03
ENSG00000162928	5194	PEX13	-0.59	8.91E-05	3.73E-03
ENSG00000005189	81691		-0.88	9.05E-05	3.74E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000137947	2959	GTF2B	-0.53	9.01E-05	3.74E-03
ENSG00000166793	219539	YPEL4	1.34	9.06E-05	3.74E-03
ENSG00000156299	7074	TIAM1	0.90	9.12E-05	3.75E-03
ENSG00000138385	6741	SSB	-0.66	9.17E-05	3.77E-03
ENSG00000231259	NA		0.99	9.22E-05	3.77E-03
ENSG00000150773	120379	PIH1D2	-1.07	9.31E-05	3.80E-03
ENSG00000172586	118487	CHCHD1	-0.80	9.32E-05	3.80E-03
ENSG00000134058	1022	CDK7	-0.78	9.49E-05	3.84E-03
ENSG00000196636	57001	SDHAF3	-0.92	9.47E-05	3.84E-03
ENSG00000116288	11315	PARK7	-0.33	9.92E-05	4.00E-03
ENSG00000078596	9452	ITM2A	1.13	1.01E-04	4.05E-03
ENSG00000092096	51310	SLC22A17	1.07	1.01E-04	4.05E-03
ENSG00000233974	NA		1.28	1.01E-04	4.05E-03
ENSG00000104419	10397	NDRG1	1.20	1.02E-04	4.07E-03
ENSG00000197993	3792	KEL	1.38	1.02E-04	4.08E-03
ENSG00000000419	8813	DPM1	-0.56	1.03E-04	4.08E-03
ENSG00000075089	64431	ACTR6	-0.66	1.04E-04	4.08E-03
ENSG00000101443	10406	WFDC2	-1.35	1.04E-04	4.08E-03
ENSG00000140526	11057	ABHD2	-0.77	1.03E-04	4.08E-03
ENSG00000198087	23607	CD2AP	-0.57	1.04E-04	4.08E-03
ENSG00000149150	8501	SLC43A1	0.96	1.05E-04	4.11E-03
ENSG00000251039	NA	IGKV2D-40	1.53	1.05E-04	4.11E-03
ENSG00000172935	116535	MRGPRF	1.56	1.06E-04	4.13E-03
ENSG00000221716	677799	SNORA11	1.44	1.06E-04	4.14E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000148400	4851	NOTCH1	0.61	1.08E-04	4.16E-03
ENSG00000224411	NA	HSP90AA2P	-0.80	1.07E-04	4.16E-03
ENSG00000103168	9013	TAF1C	0.98	1.11E-04	4.28E-03
ENSG00000032742	8100	IFT88	-0.68	1.13E-04	4.34E-03
ENSG00000112297	202	AIM1	-0.48	1.15E-04	4.42E-03
ENSG00000172037	3913	LAMB2	0.72	1.15E-04	4.42E-03
ENSG00000119328	54942	FAM206A	-0.63	1.17E-04	4.45E-03
ENSG00000164114	79884	MAP9	-0.78	1.17E-04	4.45E-03
ENSG00000100591	10598	AHSA1	-0.74	1.22E-04	4.62E-03
ENSG00000127884	1892	ECHS1	-0.42	1.24E-04	4.62E-03
ENSG00000134709	51361	HOOK1	-0.81	1.23E-04	4.62E-03
ENSG00000153904	23576	DDAH1	-0.53	1.22E-04	4.62E-03
ENSG00000165929	123036	TC2N	-0.81	1.24E-04	4.62E-03
ENSG00000169550	143662	MUC15	-1.09	1.23E-04	4.62E-03
ENSG00000214776	NA		1.28	1.23E-04	4.62E-03
ENSG00000163406	6565	SLC15A2	-0.97	1.24E-04	4.63E-03
ENSG00000177054	54503	ZDHHC13	-0.84	1.24E-04	4.63E-03
ENSG00000100626	57452	GALNT16	1.34	1.27E-04	4.70E-03
ENSG00000129055	25847	ANAPC13	-0.81	1.27E-04	4.70E-03
ENSG00000080824	3320	HSP90AA1	-0.67	1.28E-04	4.72E-03
ENSG00000119912	3416	IDE	-0.54	1.31E-04	4.81E-03
ENSG00000148346	3934	LCN2	-1.55	1.31E-04	4.82E-03
ENSG00000211459	NA	MT-RNR1	0.83	1.32E-04	4.85E-03
ENSG00000175309	85007	PHYKPL	0.77	1.33E-04	4.87E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000178460	157777	MCMDC2	-1.36	1.33E-04	4.87E-03
ENSG00000159079	56683	C21orf59	-1.02	1.37E-04	4.99E-03
ENSG00000137691	85016	C11orf70	-1.02	1.38E-04	5.02E-03
ENSG00000185585	169611	OLFML2A	1.02	1.39E-04	5.02E-03
ENSG00000127838	25953	PNKD	-0.49	1.40E-04	5.06E-03
ENSG00000143387	1513	CTSK	1.44	1.40E-04	5.06E-03
ENSG00000142733	9064	MAP3K6	0.60	1.42E-04	5.11E-03
ENSG00000147862	4781	NFIB	0.61	1.46E-04	5.22E-03
ENSG00000115339	2591	GALNT3	-0.90	1.46E-04	5.23E-03
ENSG00000073969	4905	NSF	-0.51	1.48E-04	5.28E-03
ENSG00000109971	3312	HSPA8	-0.53	1.49E-04	5.29E-03
ENSG00000116299	57535	KIAA1324	-0.76	1.51E-04	5.35E-03
ENSG00000092421	57556	SEMA6A	1.00	1.52E-04	5.36E-03
ENSG00000129493	25938	HEATR5A	-0.39	1.52E-04	5.36E-03
ENSG00000197279	7718	ZNF165	-0.98	1.51E-04	5.36E-03
ENSG00000161544	114757	CYGB	0.93	1.54E-04	5.41E-03
ENSG00000211890	NA	IGHA2	1.70	1.54E-04	5.42E-03
ENSG00000133063	1118	CHIT1	1.69	1.57E-04	5.50E-03
ENSG00000160469	84446	BRSK1	1.36	1.62E-04	5.63E-03
ENSG00000185681	254956	MORN5	-1.16	1.61E-04	5.63E-03
ENSG00000122420	5737	PTGFR	-1.33	1.63E-04	5.66E-03
ENSG00000130066	6303	SAT1	-0.63	1.63E-04	5.66E-03
ENSG00000179222	9500	MAGED1	-0.58	1.65E-04	5.72E-03
ENSG00000095380	54187	NANS	-0.75	1.68E-04	5.79E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000089127	4938	OAS1	-1.31	1.69E-04	5.83E-03
ENSG00000066185	84217	ZMYND12	-1.04	1.70E-04	5.83E-03
ENSG00000234745	3106	HLA-B	-0.84	1.71E-04	5.85E-03
ENSG00000175792	8607	RUVBL1	-0.80	1.72E-04	5.88E-03
ENSG00000101846	412	STS	-0.54	1.75E-04	5.97E-03
ENSG00000206149	440248	HERC2P9	0.90	1.78E-04	6.06E-03
ENSG00000076685	22978	NT5C2	-0.42	1.79E-04	6.09E-03
ENSG00000069702	7049	TGFBR3	0.94	1.80E-04	6.10E-03
ENSG00000153292	266977	ADGRF1	-1.45	1.81E-04	6.11E-03
ENSG00000059145	64718	UNKL	0.91	1.83E-04	6.14E-03
ENSG00000113212	56129	PCDHB7	1.36	1.83E-04	6.14E-03
ENSG00000177455	930	CD19	1.71	1.82E-04	6.14E-03
ENSG00000106483	6424	SFRP4	1.62	1.83E-04	6.14E-03
ENSG00000013016	30845	EHD3	1.11	1.85E-04	6.18E-03
ENSG00000168961	3965	LGALS9	-0.70	1.85E-04	6.18E-03
ENSG00000163131	1520	CTSS	-0.77	1.87E-04	6.24E-03
ENSG00000147041	94122	SYTL5	-1.12	1.89E-04	6.25E-03
ENSG00000214517	51400	PPME1	-0.60	1.89E-04	6.25E-03
ENSG00000211956	NA	IGHV4-34	1.68	1.89E-04	6.26E-03
ENSG00000110675	55531	ELMOD1	1.58	1.91E-04	6.29E-03
ENSG00000128039	79644	SRD5A3	-0.97	1.93E-04	6.35E-03
ENSG00000185813	5833	PCYT2	-0.92	1.95E-04	6.40E-03
ENSG00000184254	220	ALDH1A3	-0.68	2.01E-04	6.58E-03
ENSG00000084207	2950	GSTP1	-0.85	2.03E-04	6.59E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000123243	80760	ITIH5	1.40	2.03E-04	6.59E-03
ENSG00000143106	5686	PSMA5	-0.56	2.02E-04	6.59E-03
ENSG00000167775	51293	CD320	0.85	2.03E-04	6.59E-03
ENSG00000187391	9863	MAGI2	0.92	2.03E-04	6.59E-03
ENSG00000168477	7148	TNXB	1.50	2.08E-04	6.74E-03
ENSG00000241755	NA	IGKV1-9	1.66	2.11E-04	6.81E-03
ENSG00000136810	7295	TXN	-0.77	2.13E-04	6.87E-03
ENSG00000104332	6422	SFRP1	1.68	2.14E-04	6.87E-03
ENSG00000184076	29796	UQCR10	-0.78	2.14E-04	6.87E-03
ENSG00000106537	27075	TSPAN13	-0.82	2.15E-04	6.88E-03
ENSG00000127362	50831	TAS2R3	1.02	2.16E-04	6.91E-03
ENSG00000160213	1476	CSTB	-0.51	2.17E-04	6.91E-03
ENSG00000067064	3422	IDI1	-0.95	2.19E-04	6.95E-03
ENSG00000143196	1805	DPT	1.57	2.21E-04	7.00E-03
ENSG00000105929	50617	ATP6V0A4	-1.54	2.24E-04	7.09E-03
ENSG00000145391	80854	SETD7	0.69	2.27E-04	7.16E-03
ENSG00000066735	26153	KIF26A	1.55	2.28E-04	7.19E-03
ENSG00000108179	10105	PPIF	-0.65	2.30E-04	7.22E-03
ENSG00000124107	6590	SLPI	-1.05	2.30E-04	7.22E-03
ENSG00000163902	6184	RPN1	-0.42	2.31E-04	7.22E-03
ENSG00000198919	9666	DZIP3	-0.79	2.31E-04	7.22E-03
ENSG00000134363	10468	FST	1.30	2.32E-04	7.24E-03
ENSG00000133328	54979	HRASLS2	-1.41	2.35E-04	7.32E-03
ENSG00000100968	4776	NFATC4	1.14	2.36E-04	7.33E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000097007	25	ABL1	0.38	2.41E-04	7.46E-03
ENSG00000147155	10682	EBP	-0.75	2.41E-04	7.46E-03
ENSG00000184432	9276	COPB2	-0.48	2.43E-04	7.50E-03
ENSG00000135387	4076	CAPRIN1	-0.38	2.44E-04	7.52E-03
ENSG00000177169	8408	ULK1	0.49	2.47E-04	7.58E-03
ENSG00000086232	27102	EIF2AK1	-0.46	2.49E-04	7.63E-03
ENSG00000132141	10693	CCT6B	-0.92	2.50E-04	7.66E-03
ENSG00000102900	9688	NUP93	-0.43	2.51E-04	7.66E-03
ENSG00000117528	5825	ABCD3	-0.57	2.52E-04	7.67E-03
ENSG00000167523	124045	SPATA33	-0.90	2.54E-04	7.74E-03
ENSG00000203734	345930	ECT2L	-0.91	2.60E-04	7.88E-03
ENSG00000044115	1495	CTNNA1	-0.40	2.65E-04	8.04E-03
ENSG00000065361	2065	ERBB3	-0.86	2.67E-04	8.06E-03
ENSG00000138294	NA	NA	-1.54	2.67E-04	8.06E-03
ENSG00000197697	NA	NA	-0.77	2.68E-04	8.07E-03
ENSG00000184983	4700	NDUFA6	-0.52	2.69E-04	8.07E-03
ENSG00000155254	83742	MARVELD1	1.25	2.73E-04	8.17E-03
ENSG00000081760	65985	AACS	-0.64	2.74E-04	8.17E-03
ENSG00000132746	222	ALDH3B2	-1.46	2.74E-04	8.17E-03
ENSG00000151364	65987	KCTD14	-1.25	2.73E-04	8.17E-03
ENSG00000164347	84340	GFM2	-0.86	2.75E-04	8.17E-03
ENSG00000105875	29062	WDR91	0.65	2.77E-04	8.21E-03
ENSG00000136153	4008	LMO7	-0.80	2.81E-04	8.31E-03
ENSG00000116133	1718	DHCR24	-0.76	2.83E-04	8.35E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000124201	57169	ZNFX1	-0.52	2.84E-04	8.37E-03
ENSG00000158234	55179	FAIM	-0.82	2.85E-04	8.38E-03
ENSG00000144834	29114	TAGLN3	-1.37	2.86E-04	8.41E-03
ENSG00000099290	387680	FAM21A	-1.43	2.87E-04	8.42E-03
ENSG00000149021	7356	SCGB1A1	-1.45	2.90E-04	8.49E-03
ENSG00000135378	79056	PRRG4	-0.92	2.92E-04	8.54E-03
ENSG00000134744	23318	ZCCHC11	0.50	2.99E-04	8.71E-03
ENSG00000005175	79657	RPAP3	-0.38	3.01E-04	8.77E-03
ENSG00000173467	155465	AGR3	-0.78	3.04E-04	8.81E-03
ENSG00000164251	2150	F2RL1	-0.87	3.12E-04	9.03E-03
ENSG00000253250	100127983	C8orf88	1.46	3.14E-04	9.09E-03
ENSG00000090061	8812	CCNK	-0.34	3.23E-04	9.32E-03
ENSG00000101204	1137	CHRNA4	1.64	3.23E-04	9.32E-03
ENSG00000143127	8515	ITGA10	1.00	3.26E-04	9.36E-03
ENSG00000187800	375033	PEAR1	1.54	3.29E-04	9.45E-03
ENSG00000162909	824	CAPN2	-0.49	3.32E-04	9.51E-03
ENSG00000103316	1428	CRYM	-1.06	3.34E-04	9.55E-03
ENSG00000069869	4734	NEDD4	0.92	3.37E-04	9.58E-03
ENSG00000162407	8613	PPAP2B	1.12	3.37E-04	9.58E-03
ENSG00000143933	805	CALM2	-0.58	3.40E-04	9.66E-03
ENSG00000125827	56255	TMX4	0.60	3.41E-04	9.67E-03
ENSG00000185055	NA	EFCAB10	-1.13	3.47E-04	9.82E-03
ENSG00000029993	3149	HMGB3	-0.96	3.54E-04	9.94E-03
ENSG00000135424	3679	ITGA7	1.29	3.53E-04	9.94E-03

Table 10. Non-smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	P value	FDR P value
ENSG00000158373	3017	HIST1H2BD	-0.54	3.52E-04	9.94E-03
ENSG00000165097	221656	KDM1B	-0.71	3.53E-04	9.94E-03
ENSG00000173432	6288	SAA1	-1.61	3.55E-04	9.95E-03

UIP (n=3 samples); Non-UIP (n=5 samples). Positive log2 fold change value indicates over-expression in UIP relative to Non UIP; negative log2 value indicates under-expression in UIP relative to Non UIP. In this analysis only patients without any smoking history were evaluated, hence this subset harbored only non-smokers.

[0186] Table 11. Differentially expressed genes in UIP samples from smokers vs. Non-UIP samples from smokers.

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000137968	204962	SLC44A5	3.58	2.38E-20	3.68E-16
ENSG00000099968	23786	BCL2L13	-0.68	3.12E-18	2.41E-14
ENSG00000168329	1524	CX3CR1	2.27	8.45E-14	4.35E-10
ENSG00000088882	56265	CPXM1	2.72	1.16E-11	4.49E-08
ENSG00000152672	165530	CLEC4F	2.63	1.78E-11	5.49E-08
ENSG00000129204	9098	USP6	2.74	5.40E-11	1.39E-07
ENSG00000132823	51526	OSER1	-0.56	3.27E-10	7.21E-07
ENSG00000177666	57104	PNPLA2	-0.84	9.60E-10	1.85E-06
ENSG00000125730	718	C3	1.60	1.11E-09	1.90E-06
ENSG00000198074	57016	AKR1B10	-2.89	1.97E-09	3.04E-06
ENSG00000198142	65124	SOWAHC	-1.00	3.09E-09	4.34E-06
ENSG00000112130	9025	RNF8	-0.56	4.53E-09	5.83E-06
ENSG00000255112	57132	CHMP1B	-0.86	1.94E-08	2.31E-05
ENSG00000145888	2741	GLRA1	2.19	2.15E-08	2.37E-05

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000151632	1646	AKR1C2	-2.37	2.60E-08	2.68E-05
ENSG00000238741	677767	SCARNA7	0.58	2.90E-08	2.80E-05
ENSG00000148948	57689	LRRC4C	2.14	3.13E-08	2.84E-05
ENSG00000179344	3119	HLA-DQB1	2.30	2.89E-07	2.35E-04
ENSG00000211789	NA	TRAV12-2	2.44	2.75E-07	2.35E-04
ENSG00000106565	28959	TMEM176B	1.55	3.23E-07	2.44E-04
ENSG00000204338	NA	CYP21A1P	2.23	3.32E-07	2.44E-04
ENSG00000010932	2326	FMO1	2.17	4.30E-07	3.01E-04
ENSG00000103742	57722	IGDCC4	2.37	4.82E-07	3.24E-04
ENSG00000162692	7412	VCAM1	2.12	5.91E-07	3.80E-04
ENSG00000158481	911	CD1C	1.96	6.26E-07	3.87E-04
ENSG00000136098	4752	NEK3	0.67	6.66E-07	3.95E-04
ENSG00000134375	10440	TIMM17A	-0.48	7.00E-07	4.00E-04
ENSG00000127951	10875	FGL2	1.23	1.18E-06	6.49E-04
ENSG00000185022	23764	MAFF	-1.68	1.22E-06	6.49E-04
ENSG00000100079	3957	LGALS2	1.93	1.51E-06	7.56E-04
ENSG00000151572	121601	ANO4	2.28	1.57E-06	7.56E-04
ENSG00000238460	NA		1.65	1.52E-06	7.56E-04
ENSG00000187527	344905	ATP13A5	-2.18	1.99E-06	9.29E-04
ENSG00000176153	2877	GPX2	-2.11	2.29E-06	1.04E-03
ENSG00000105559	57664	PLEKHA4	1.30	2.99E-06	1.32E-03
ENSG00000178115	NA	GOLGA8Q	2.05	3.27E-06	1.40E-03
ENSG00000137033	90865	IL33	1.41	4.10E-06	1.68E-03
ENSG00000196735	3117	HLA-DQA1	2.10	4.14E-06	1.68E-03

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000007944	29116	MYLIP	-0.46	4.53E-06	1.79E-03
ENSG00000130695	64793	CEP85	-0.76	4.86E-06	1.88E-03
ENSG00000262539	NA		-2.01	5.01E-06	1.89E-03
ENSG00000174194	NA	NA	1.27	5.42E-06	1.99E-03
ENSG00000178187	285676	ZNF454	1.08	6.40E-06	2.25E-03
ENSG00000204256	6046	BRD2	-0.33	6.28E-06	2.25E-03
ENSG00000002933	55365	TMEM176A	1.30	6.91E-06	2.36E-03
ENSG00000196139	8644	AKR1C3	-1.45	7.02E-06	2.36E-03
ENSG00000186529	4051	CYP4F3	-1.80	8.32E-06	2.66E-03
ENSG00000227097	NA	RPS28P7	1.59	8.45E-06	2.66E-03
ENSG00000244486	91179	SCARF2	1.00	8.18E-06	2.66E-03
ENSG00000172985	344558	SH3RF3	0.84	9.51E-06	2.94E-03
ENSG00000023171	57476	GRAMD1B	-1.22	1.13E-05	3.21E-03
ENSG00000065613	9748	SLK	-0.49	1.09E-05	3.21E-03
ENSG00000143603	3782	KCNN3	1.09	1.07E-05	3.21E-03
ENSG00000154096	7070	THY1	2.05	1.14E-05	3.21E-03
ENSG00000178562	940	CD28	2.08	1.11E-05	3.21E-03
ENSG00000196839	100	ADA	1.10	1.16E-05	3.21E-03
ENSG00000159618	221188	ADGRG5	1.59	1.21E-05	3.27E-03
ENSG00000128309	4357	MPST	-0.73	1.36E-05	3.61E-03
ENSG00000168229	5729	PTGDR	1.61	1.39E-05	3.62E-03
ENSG00000125510	4987	OPRL1	1.56	1.45E-05	3.73E-03
ENSG00000017427	3479	IGF1	1.86	1.54E-05	3.83E-03
ENSG00000159228	873	CBR1	-1.00	1.53E-05	3.83E-03

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000136490	80774	LIMD2	1.68	1.76E-05	4.19E-03
ENSG00000177156	6888	TALDO1	-0.78	1.76E-05	4.19E-03
ENSG00000225614	84627	ZNF469	0.98	1.72E-05	4.19E-03
ENSG00000218336	55714	TENM3	1.69	1.88E-05	4.40E-03
ENSG00000151693	8853	ASAP2	-0.60	1.97E-05	4.54E-03
ENSG00000211941	NA	IGHV3-11	2.04	2.00E-05	4.54E-03
ENSG00000140961	29948	OSGIN1	-1.25	2.11E-05	4.73E-03
ENSG00000124782	6239	RREB1	-0.41	2.30E-05	5.08E-03
ENSG00000103222	4363	ABCC1	-0.81	2.55E-05	5.47E-03
ENSG00000196664	51284	TLR7	1.53	2.54E-05	5.47E-03
ENSG00000148357	256158	HMCN2	1.92	2.61E-05	5.53E-03
ENSG00000124151	8202	NCOA3	-0.39	2.87E-05	6.00E-03
ENSG00000211653	NA	IGLV1-40	1.99	2.98E-05	6.06E-03
ENSG00000230006	645784	ANKRD36BP2	1.82	2.97E-05	6.06E-03
ENSG00000106809	4969	OGN	1.85	3.11E-05	6.23E-03
ENSG00000162877	148811	PM20D1	1.70	3.17E-05	6.28E-03
ENSG00000128016	7538	ZFP36	-1.41	3.51E-05	6.77E-03
ENSG00000196345	55888	ZKSCAN7	0.83	3.49E-05	6.77E-03
ENSG00000108821	1277	COL1A1	1.88	3.56E-05	6.78E-03
ENSG00000137573	23213	SULF1	1.63	3.68E-05	6.93E-03
ENSG00000197993	3792	KEL	1.86	3.93E-05	7.32E-03
ENSG00000170153	57484	RNF150	1.44	4.36E-05	8.00E-03
ENSG00000130513	9518	GDF15	-1.73	4.55E-05	8.20E-03
ENSG00000174123	81793	TLR10	1.81	4.57E-05	8.20E-03

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000110076	9379	NRXN2	1.95	4.68E-05	8.30E-03
ENSG00000182551	55256	ADI1	-0.52	5.20E-05	9.08E-03
ENSG00000182557	201305	SPNS3	1.65	5.23E-05	9.08E-03
ENSG00000117215	26279	PLA2G2D	1.94	5.54E-05	9.50E-03
ENSG00000128285	2847	MCHR1	1.85	5.80E-05	9.83E-03
ENSG00000183813	1233	CCR4	1.90	6.00E-05	1.01E-02
ENSG00000007312	974	CD79B	1.81	6.30E-05	1.04E-02
ENSG00000163817	54716	SLC6A20	1.85	6.43E-05	1.06E-02
ENSG00000102802	84935	MEDAG	1.85	6.69E-05	1.09E-02
ENSG00000101134	55816	DOK5	1.90	7.00E-05	1.10E-02
ENSG00000102362	94121	SYTL4	-0.80	6.89E-05	1.10E-02
ENSG00000128000	163131	ZNF780B	0.66	7.03E-05	1.10E-02
ENSG00000256229	90649	ZNF486	1.00	7.04E-05	1.10E-02
ENSG00000086102	4799	NFX1	-0.36	7.46E-05	1.14E-02
ENSG00000099875	2872	MKNK2	-0.58	7.58E-05	1.14E-02
ENSG00000171502	255631	COL24A1	1.24	7.69E-05	1.14E-02
ENSG00000211637	NA	IGLV4-69	1.91	7.66E-05	1.14E-02
ENSG00000244731	720	C4A	1.50	7.70E-05	1.14E-02
ENSG00000082641	4779	NFE2L1	-0.32	7.79E-05	1.14E-02
ENSG00000136802	56262	LRRC8A	-0.91	8.12E-05	1.18E-02
ENSG00000225784	NA	NA	1.81	8.26E-05	1.19E-02
ENSG00000181631	53829	P2RY13	1.57	8.40E-05	1.20E-02
ENSG00000116031	50489	CD207	1.47	8.80E-05	1.25E-02
ENSG00000108106	27338	UBE2S	-0.71	9.40E-05	1.32E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000105639	3718	JAK3	1.60	1.06E-04	1.43E-02
ENSG00000125804	284800	FAM182A	1.82	1.07E-04	1.43E-02
ENSG00000139722	79720	VPS37B	-0.67	1.07E-04	1.43E-02
ENSG00000157557	2114	ETS2	-0.85	1.03E-04	1.43E-02
ENSG00000165841	1557	CYP2C19	-1.37	1.07E-04	1.43E-02
ENSG00000182487	654816	NCF1B	1.59	1.05E-04	1.43E-02
ENSG00000171847	55138	FAM90A1	1.18	1.08E-04	1.43E-02
ENSG00000162804	25992	SNED1	0.71	1.09E-04	1.43E-02
ENSG00000186184	51082	POLR1D	-0.67	1.11E-04	1.43E-02
ENSG00000172493	4299	AFF1	-0.34	1.14E-04	1.47E-02
ENSG00000179954	284297	SSC5D	1.43	1.15E-04	1.47E-02
ENSG00000244682	NA	FCGR2C	1.54	1.17E-04	1.48E-02
ENSG00000081148	50939	IMPG2	0.85	1.23E-04	1.54E-02
ENSG00000126353	1236	CCR7	1.51	1.26E-04	1.57E-02
ENSG00000197705	57565	KLHL14	1.75	1.27E-04	1.57E-02
ENSG00000232268	390037	OR52I1	1.53	1.30E-04	1.59E-02
ENSG00000099204	3983	ABLIM1	-0.69	1.32E-04	1.61E-02
ENSG00000158477	909	CD1A	1.84	1.39E-04	1.68E-02
ENSG00000172336	10248	POP7	-0.45	1.40E-04	1.68E-02
ENSG00000138109	1559	CYP2C9	-1.47	1.44E-04	1.71E-02
ENSG00000124766	6659	SOX4	-0.89	1.50E-04	1.77E-02
ENSG00000114115	5947	RBP1	0.96	1.57E-04	1.84E-02
ENSG00000156463	153769	SH3RF2	-1.28	1.62E-04	1.88E-02
ENSG00000038358	23644	EDC4	-0.26	1.64E-04	1.88E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000211899	NA	IGHM	1.79	1.64E-04	1.88E-02
ENSG00000263503	NA		-1.70	1.66E-04	1.88E-02
ENSG00000005955	NA	NA	-0.32	1.71E-04	1.91E-02
ENSG00000144040	94097	SFXN5	0.69	1.71E-04	1.91E-02
ENSG00000105058	26017	FAM32A	-0.37	1.90E-04	2.11E-02
ENSG00000135837	9857	CEP350	-0.29	1.92E-04	2.12E-02
ENSG00000205148	NA	NA	1.63	1.93E-04	2.12E-02
ENSG00000028277	5452	POU2F2	1.61	1.97E-04	2.15E-02
ENSG00000211747	NA	TRBV20-1	1.78	2.04E-04	2.20E-02
ENSG00000148730	1979	EIF4EBP2	-0.51	2.25E-04	2.40E-02
ENSG00000181036	343413	FCRL6	1.52	2.26E-04	2.40E-02
ENSG00000138660	55435	AP1AR	-0.61	2.29E-04	2.41E-02
ENSG00000144619	152330	CNTN4	1.25	2.28E-04	2.41E-02
ENSG00000000971	3075	CFH	0.81	2.42E-04	2.46E-02
ENSG00000099251	158160	HSD17B7P2	0.95	2.44E-04	2.46E-02
ENSG00000156140	9508	ADAMTS3	1.24	2.42E-04	2.46E-02
ENSG00000163113	NA	NA	-0.63	2.41E-04	2.46E-02
ENSG00000181458	55076	TMEM45A	1.40	2.37E-04	2.46E-02
ENSG00000251287	644974	ALG1L2	1.42	2.44E-04	2.46E-02
ENSG00000086544	80271	ITPKC	-0.57	2.52E-04	2.52E-02
ENSG00000167984	197358	NLRC3	1.07	2.55E-04	2.54E-02
ENSG00000149633	85449	KIAA1755	1.69	2.59E-04	2.56E-02
ENSG00000102271	56062	KLHL4	1.75	2.62E-04	2.58E-02
ENSG00000137815	23168	RTF1	-0.36	2.81E-04	2.72E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000141682	5366	PMAIP1	-1.36	2.81E-04	2.72E-02
ENSG00000179909	7710	ZNF154	1.14	2.80E-04	2.72E-02
ENSG00000110427	25758	KIAA1549L	1.52	2.89E-04	2.77E-02
ENSG00000144567	79137	FAM134A	-0.43	2.97E-04	2.83E-02
ENSG00000206561	8292	COLQ	0.81	3.01E-04	2.85E-02
ENSG00000100304	23170	TTLL12	-0.92	3.11E-04	2.86E-02
ENSG00000108852	4355	MPP2	1.61	3.07E-04	2.86E-02
ENSG00000181350	388341	LRRC75A	1.56	3.11E-04	2.86E-02
ENSG00000186350	6256	RXRA	-0.53	3.08E-04	2.86E-02
ENSG00000253816	NA		0.93	3.05E-04	2.86E-02
ENSG00000026751	57823	SLAMF7	1.36	3.13E-04	2.86E-02
ENSG00000142178	150094	SIK1	-1.46	3.17E-04	2.86E-02
ENSG00000148848	8038	ADAM12	1.19	3.16E-04	2.86E-02
ENSG00000148339	114789	SLC25A25	-0.71	3.20E-04	2.88E-02
ENSG00000137747	84000	TMPRSS13	-0.89	3.23E-04	2.88E-02
ENSG00000164061	8927	BSN	1.16	3.29E-04	2.92E-02
ENSG00000102221	9767	JADE3	-0.48	3.34E-04	2.95E-02
ENSG00000134291	79022	TMEM106C	-0.84	3.67E-04	3.20E-02
ENSG00000160007	2909	ARHGAP35	-0.32	3.69E-04	3.20E-02
ENSG00000198060	54708	MARCH5	-0.39	3.67E-04	3.20E-02
ENSG00000111725	5564	PRKAB1	-0.72	3.71E-04	3.20E-02
ENSG00000211598	NA	IGKV4-1	1.72	3.76E-04	3.23E-02
ENSG00000101096	4773	NFATC2	0.89	3.82E-04	3.25E-02
ENSG00000215156	646652		1.07	3.83E-04	3.25E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000117090	6504	SLAMF1	1.64	3.89E-04	3.26E-02
ENSG00000211892	NA	IGHG4	1.71	3.88E-04	3.26E-02
ENSG00000129245	9513	FXR2	-0.35	4.00E-04	3.33E-02
ENSG00000211685	NA	IGLC7	1.71	4.06E-04	3.37E-02
ENSG00000085265	2219	FCN1	1.67	4.11E-04	3.38E-02
ENSG00000108691	6347	CCL2	1.64	4.16E-04	3.38E-02
ENSG00000109787	51274	KLF3	-0.67	4.15E-04	3.38E-02
ENSG00000243264	NA	IGKV2D-29	1.71	4.13E-04	3.38E-02
ENSG00000178199	340152	ZC3H12D	1.65	4.19E-04	3.38E-02
ENSG00000152413	9456	HOMER1	-0.58	4.24E-04	3.39E-02
ENSG00000211893	NA	IGHG2	1.70	4.25E-04	3.39E-02
ENSG00000241755	NA	IGKV1-9	1.70	4.21E-04	3.39E-02
ENSG00000017797	10928	RALBP1	-0.39	4.36E-04	3.44E-02
ENSG00000136826	9314	KLF4	-1.16	4.37E-04	3.44E-02
ENSG00000102245	959	CD40LG	1.65	4.53E-04	3.49E-02
ENSG00000144792	285349	ZNF660	0.91	4.51E-04	3.49E-02
ENSG00000151012	23657	SLC7A11	-1.55	4.45E-04	3.49E-02
ENSG00000182218	84439	HHIPL1	1.55	4.48E-04	3.49E-02
ENSG00000204961	9752	PCDHA9	1.11	4.54E-04	3.49E-02
ENSG00000160229	NA	ZNF66	0.77	4.59E-04	3.51E-02
ENSG00000087842	8544	PIR	-0.94	4.67E-04	3.55E-02
ENSG00000108344	5709	PSMD3	-0.35	4.76E-04	3.60E-02
ENSG00000167077	150365	MEI1	1.53	4.87E-04	3.67E-02
ENSG00000211625	NA	IGKV3D-20	1.68	4.91E-04	3.68E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000087152	56970	ATXN7L3	-0.27	5.09E-04	3.80E-02
ENSG00000138166	1847	DUSP5	-1.57	5.15E-04	3.82E-02
ENSG00000187987	222696	ZSCAN23	1.19	5.20E-04	3.84E-02
ENSG00000166676	780776	TVP23A	1.60	5.24E-04	3.85E-02
ENSG00000107736	64072	CDH23	1.28	5.27E-04	3.86E-02
ENSG00000109685	7468	WHSC1	-0.43	5.41E-04	3.93E-02
ENSG00000240382	NA	IGKV1-17	1.67	5.43E-04	3.93E-02
ENSG00000196724	147686	ZNF418	0.90	5.47E-04	3.95E-02
ENSG00000178031	92949	ADAMTSL1	1.64	5.54E-04	3.98E-02
ENSG00000109854	10553	HTATIP2	-0.72	5.69E-04	4.03E-02
ENSG00000128606	10234	LRRC17	1.37	5.69E-04	4.03E-02
ENSG00000211974	NA		1.66	5.69E-04	4.03E-02
ENSG00000145002	653333	FAM86B2	1.42	5.90E-04	4.14E-02
ENSG00000185271	123103	KLHL33	1.66	5.88E-04	4.14E-02
ENSG00000147394	7739	ZNF185	-0.95	6.04E-04	4.22E-02
ENSG00000105369	973	CD79A	1.65	6.09E-04	4.23E-02
ENSG00000205403	3426	CFI	0.87	6.11E-04	4.23E-02
ENSG00000242732	340526	RGAG4	0.69	6.16E-04	4.24E-02
ENSG00000074410	771	CA12	-1.59	6.25E-04	4.29E-02
ENSG00000107020	55848	PLGRKT	0.66	6.34E-04	4.31E-02
ENSG00000145555	4651	MYO10	-0.45	6.31E-04	4.31E-02
ENSG00000183486	4600	MX2	-1.26	6.46E-04	4.37E-02
ENSG00000066827	57623	ZFAT	0.33	6.62E-04	4.46E-02
ENSG00000225217	NA	HSPA7	1.46	6.65E-04	4.46E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000143851	5778	PTPN7	1.35	6.77E-04	4.50E-02
ENSG00000161381	57125	PLXDC1	1.44	6.76E-04	4.50E-02
ENSG00000134775	80206	FHOD3	1.24	6.89E-04	4.55E-02
ENSG00000154040	26256	CABYR	-1.18	6.89E-04	4.55E-02
ENSG00000239951	NA	IGKV3-20	1.64	6.97E-04	4.58E-02
ENSG00000137709	25833	POU2F3	-0.82	7.04E-04	4.60E-02
ENSG00000123159	10755	GIPC1	-0.48	7.11E-04	4.61E-02
ENSG00000211939	NA	NA	1.63	7.10E-04	4.61E-02
ENSG00000211666	NA	IGLV2-14	1.63	7.17E-04	4.63E-02
ENSG00000227507	4050	LTB	1.60	7.24E-04	4.66E-02
ENSG00000171714	203859	ANO5	1.12	7.41E-04	4.75E-02
ENSG00000078589	27334	P2RY10	1.38	7.53E-04	4.76E-02
ENSG00000108381	443	ASPA	1.44	7.50E-04	4.76E-02
ENSG00000186310	4675	NAP1L3	1.58	7.47E-04	4.76E-02
ENSG00000164398	23305	ACSL6	1.19	7.61E-04	4.80E-02
ENSG00000117122	4237	MFAP2	1.28	7.65E-04	4.80E-02
ENSG00000145536	170690	ADAMTS16	1.62	7.71E-04	4.80E-02
ENSG00000251402	NA	FAM90A25P	1.02	7.70E-04	4.80E-02
ENSG00000161681	50944	SHANK1	1.62	7.95E-04	4.93E-02
ENSG00000130584	140685	ZBTB46	0.99	8.00E-04	4.93E-02
ENSG00000204839	642475	MROH6	-1.17	8.02E-04	4.93E-02
ENSG00000204544	394263	MUC21	-1.52	8.06E-04	4.94E-02
ENSG00000112245	7803	PTP4A1	-0.59	8.14E-04	4.97E-02
ENSG00000137265	3662	IRF4	1.61	8.28E-04	5.04E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000105364	51073	MRPL4	-0.56	8.39E-04	5.06E-02
ENSG00000178922	81888	HYI	0.84	8.36E-04	5.06E-02
ENSG00000118849	5918	RARRES1	1.24	8.47E-04	5.09E-02
ENSG00000212743	NA		1.23	8.58E-04	5.13E-02
ENSG00000135074	8728	ADAM19	1.42	8.76E-04	5.16E-02
ENSG00000152689	25780	RASGRP3	1.42	8.72E-04	5.16E-02
ENSG00000154640	10950	BTG3	-0.77	8.74E-04	5.16E-02
ENSG00000196581	55966	AJAP1	-1.59	8.74E-04	5.16E-02
ENSG00000211950	NA	IGHV1-24	1.60	9.04E-04	5.30E-02
ENSG00000188171	199777	ZNF626	0.72	9.17E-04	5.36E-02
ENSG00000134490	85019	TMEM241	0.50	9.27E-04	5.40E-02
ENSG00000186854	129293	TRABD2A	1.13	9.34E-04	5.42E-02
ENSG00000173198	10800	CYSLTR1	1.16	9.39E-04	5.43E-02
ENSG00000132669	54453	RIN2	-0.47	9.54E-04	5.48E-02
ENSG00000211640	NA	IGLV6-57	1.59	9.52E-04	5.48E-02
ENSG00000159450	7062	TCHH	-1.34	9.59E-04	5.48E-02
ENSG00000143515	57198	ATP8B2	0.79	9.73E-04	5.54E-02
ENSG00000198734	2153	F5	1.10	9.90E-04	5.62E-02
ENSG00000229645	NA	NA	1.32	9.99E-04	5.65E-02
ENSG00000106333	5118	PCOLCE	1.21	1.02E-03	5.66E-02
ENSG00000166869	63928	CHP2	1.49	1.01E-03	5.66E-02
ENSG00000243238	NA	IGKV2-30	1.59	1.02E-03	5.66E-02
ENSG00000259236	NA	GOLGA8VP	1.52	1.01E-03	5.66E-02
ENSG00000240864	NA	IGKV1-16	1.59	1.02E-03	5.67E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000170509	345275	HSD17B13	1.49	1.03E-03	5.68E-02
ENSG00000235602	642559	POU5F1P3	1.51	1.06E-03	5.84E-02
ENSG00000125813	5075	PAX1	1.57	1.10E-03	6.04E-02
ENSG00000138413	3417	IDH1	-0.69	1.11E-03	6.04E-02
ENSG00000161896	117283	IP6K3	-1.20	1.11E-03	6.04E-02
ENSG00000183624	56941	HMCES	-0.49	1.11E-03	6.04E-02
ENSG00000196834	653269	POTEI	1.56	1.11E-03	6.04E-02
ENSG00000110777	5450	POU2AF1	1.42	1.14E-03	6.10E-02
ENSG00000183773	150209	AIFM3	1.17	1.14E-03	6.10E-02
ENSG00000225698	NA	IGHV3-72	1.57	1.13E-03	6.10E-02
ENSG00000023445	330	BIRC3	0.84	1.18E-03	6.27E-02
ENSG00000100078	50487	PLA2G3	1.42	1.18E-03	6.27E-02
ENSG00000116690	10216	PRG4	1.56	1.19E-03	6.27E-02
ENSG00000136011	55576	STAB2	1.54	1.19E-03	6.27E-02
ENSG00000172986	727936	GXYLT2	0.82	1.19E-03	6.27E-02
ENSG00000196344	131	ADH7	-1.54	1.19E-03	6.27E-02
ENSG00000197006	51108	METTL9	-0.35	1.21E-03	6.33E-02
ENSG00000087303	22795	NID2	1.37	1.22E-03	6.34E-02
ENSG00000211947	NA	IGHV3-21	1.56	1.23E-03	6.38E-02
ENSG00000170471	57148	RALGAPB	-0.27	1.23E-03	6.39E-02
ENSG00000146374	84870	RSPO3	1.55	1.25E-03	6.45E-02
ENSG00000221970	346528	OR2A1	1.49	1.26E-03	6.48E-02
ENSG00000163354	127579	DCST2	1.15	1.27E-03	6.50E-02
ENSG00000008277	53616	ADAM22	0.80	1.28E-03	6.51E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000198829	56670	SUCNR1	1.51	1.27E-03	6.51E-02
ENSG00000145808	171019	ADAMTS19	1.13	1.29E-03	6.51E-02
ENSG00000197102	1778	DYNC1H1	-0.25	1.29E-03	6.51E-02
ENSG00000198821	919	CD247	1.40	1.30E-03	6.58E-02
ENSG00000221914	5520	PPP2R2A	-0.36	1.32E-03	6.64E-02
ENSG00000078898	80341	BPIFB2	-1.47	1.36E-03	6.69E-02
ENSG00000122140	51116	MRPS2	-0.56	1.35E-03	6.69E-02
ENSG00000123595	9367	RAB9A	-0.50	1.35E-03	6.69E-02
ENSG00000145782	9140	ATG12	0.46	1.35E-03	6.69E-02
ENSG00000163297	118429	ANTXR2	0.84	1.35E-03	6.69E-02
ENSG00000180479	51276	ZNF571	0.65	1.36E-03	6.69E-02
ENSG00000182578	1436	CSF1R	1.23	1.35E-03	6.69E-02
ENSG00000185518	9899	SV2B	1.22	1.37E-03	6.71E-02
ENSG00000056586	54542	RC3H2	-0.24	1.38E-03	6.74E-02
ENSG00000021574	6683	SPAST	-0.28	1.39E-03	6.76E-02
ENSG00000008394	4257	MGST1	-0.73	1.40E-03	6.77E-02
ENSG00000112715	7422	VEGFA	-1.09	1.40E-03	6.77E-02
ENSG00000171724	57687	VAT1L	1.54	1.41E-03	6.81E-02
ENSG00000124226	55905	RNF114	-0.35	1.43E-03	6.87E-02
ENSG00000147459	80005	DOCK5	-0.44	1.45E-03	6.93E-02
ENSG00000147140	4841	NONO	-0.27	1.46E-03	6.99E-02
ENSG00000118922	11278	KLF12	0.79	1.49E-03	7.08E-02
ENSG00000142731	10733	PLK4	-1.30	1.50E-03	7.12E-02
ENSG00000159388	7832	BTG2	-1.23	1.50E-03	7.12E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000168904	123355	LRRC28	-0.39	1.53E-03	7.20E-02
ENSG00000234231	NA		1.34	1.53E-03	7.20E-02
ENSG00000187446	11261	CHP1	-0.52	1.54E-03	7.22E-02
ENSG00000180251	389015	SLC9A4	-1.51	1.55E-03	7.25E-02
ENSG00000165912	29763	PACSIN3	-0.86	1.55E-03	7.25E-02
ENSG00000165178	654817	NCF1C	1.35	1.56E-03	7.25E-02
ENSG00000128383	200315	APOBEC3A	1.48	1.59E-03	7.39E-02
ENSG00000155158	158219	TTC39B	-0.49	1.60E-03	7.40E-02
ENSG00000224041	NA	IGKV3D-15	1.51	1.62E-03	7.45E-02
ENSG00000101782	8780	RIOK3	-0.40	1.65E-03	7.55E-02
ENSG00000108671	5717	PSMD11	-0.30	1.65E-03	7.55E-02
ENSG00000135821	2752	GLUL	-0.54	1.65E-03	7.55E-02
ENSG00000076053	10179	RBM7	-0.35	1.68E-03	7.62E-02
ENSG00000122966	11113	CIT	-1.03	1.68E-03	7.62E-02
ENSG00000253755	NA	IGHGP	1.52	1.68E-03	7.62E-02
ENSG00000120129	1843	DUSP1	-1.38	1.73E-03	7.64E-02
ENSG00000121807	729230	CCR2	1.29	1.72E-03	7.64E-02
ENSG00000134046	8932	MBD2	-0.24	1.73E-03	7.64E-02
ENSG00000135773	10753	CAPN9	-1.11	1.73E-03	7.64E-02
ENSG00000136830	64855	FAM129B	-0.31	1.72E-03	7.64E-02
ENSG00000157601	4599	MX1	-1.06	1.72E-03	7.64E-02
ENSG00000162949	92291	CAPN13	0.99	1.69E-03	7.64E-02
ENSG00000173653	9986	RCE1	-0.43	1.73E-03	7.64E-02
ENSG00000177106	64787	EPS8L2	-0.65	1.73E-03	7.64E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000115590	7850	IL1R2	-1.50	1.74E-03	7.65E-02
ENSG00000136816	27348	TOR1B	-0.45	1.75E-03	7.68E-02
ENSG00000091831	2099	ESR1	1.43	1.78E-03	7.76E-02
ENSG00000101544	22850	ADNP2	-0.34	1.78E-03	7.76E-02
ENSG00000086548	4680	CEACAM6	-1.10	1.79E-03	7.78E-02
ENSG00000141738	2886	GRB7	-0.76	1.79E-03	7.78E-02
ENSG00000224078	NA	SNHG14	0.61	1.80E-03	7.78E-02
ENSG00000131408	7376	NR1H2	-0.42	1.81E-03	7.80E-02
ENSG00000111412	79794	C12orf49	-0.81	1.82E-03	7.84E-02
ENSG00000124356	10617	STAMBP	-0.33	1.85E-03	7.95E-02
ENSG00000232216	NA	IGHV3-43	1.49	1.86E-03	7.96E-02
ENSG00000115963	390	RND3	-0.89	1.90E-03	8.05E-02
ENSG00000120647	84318	CCDC77	-0.46	1.90E-03	8.05E-02
ENSG00000141540	94015	TTYH2	1.04	1.90E-03	8.05E-02
ENSG00000147234	84443	FRMPD3	1.24	1.90E-03	8.05E-02
ENSG00000189221	4128	MAOA	-1.08	1.91E-03	8.08E-02
ENSG00000125430	9953	HS3ST3B1	-0.95	1.94E-03	8.15E-02
ENSG00000144191	1261	CNGA3	1.43	1.94E-03	8.15E-02
ENSG00000132405	57533	TBC1D14	-0.40	1.95E-03	8.16E-02
ENSG00000179840	644997	PIK3CD-AS1	1.50	1.96E-03	8.16E-02
ENSG00000259261	NA	IGHV4OR15-8	1.49	1.96E-03	8.16E-02
ENSG00000147168	3561	IL2RG	1.31	1.98E-03	8.21E-02
ENSG00000164692	1278	COL1A2	1.46	2.03E-03	8.40E-02
ENSG00000013563	1774	DNASE1L1	-0.82	2.04E-03	8.42E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000103522	50615	IL21R	1.46	2.04E-03	8.42E-02
ENSG00000108091	8030	CCDC6	-0.30	2.11E-03	8.64E-02
ENSG00000157954	26100	WIPI2	-0.40	2.11E-03	8.64E-02
ENSG00000070214	23446	SLC44A1	-0.42	2.13E-03	8.70E-02
ENSG00000156671	142891	SAMD8	-0.39	2.17E-03	8.80E-02
ENSG00000174807	57124	CD248	1.46	2.17E-03	8.80E-02
ENSG00000154277	7345	UCHL1	-1.35	2.19E-03	8.82E-02
ENSG00000157766	176	ACAN	1.48	2.18E-03	8.82E-02
ENSG00000171475	147179	WIPF2	-0.44	2.19E-03	8.82E-02
ENSG00000211945	NA	IGHV1-18	1.48	2.19E-03	8.82E-02
ENSG00000186407	342510	CD300E	1.46	2.21E-03	8.86E-02
ENSG00000211659	NA	IGLV3-25	1.48	2.21E-03	8.86E-02
ENSG00000167797	10263	CDK2AP2	-0.53	2.24E-03	8.92E-02
ENSG00000132465	3512	JCHAIN	1.47	2.25E-03	8.95E-02
ENSG00000201643	677801	SNORA14A	0.93	2.25E-03	8.95E-02
ENSG00000036530	10858	CYP46A1	1.15	2.26E-03	8.96E-02
ENSG00000162782	163589	TDRD5	1.42	2.27E-03	8.96E-02
ENSG00000224373	NA	IGHV4-59	1.47	2.28E-03	8.96E-02
ENSG00000132938	23281	MTUS2	1.44	2.28E-03	8.96E-02
ENSG00000112299	8876	VNN1	1.37	2.35E-03	9.19E-02
ENSG00000164100	9348	NDST3	1.12	2.36E-03	9.22E-02
ENSG00000165949	3429	IFI27	-1.30	2.36E-03	9.22E-02
ENSG00000107643	5599	MAPK8	-0.33	2.39E-03	9.29E-02
ENSG00000184481	4303	FOXO4	-0.55	2.40E-03	9.29E-02

Table 11. Smokers Ensembl ID	Entrez ID	Gene symbol	Log2 Fold Change (UIP/NonUIP)	p value	FDR P value
ENSG00000105122	64926	RASAL3	1.32	2.43E-03	9.40E-02
ENSG00000167100	201191	SAMD14	1.44	2.44E-03	9.42E-02
ENSG00000198848	1066	CES1	-1.10	2.51E-03	9.63E-02
ENSG00000211964	NA	IGHV3-48	1.45	2.50E-03	9.63E-02
ENSG00000099958	91319	DERL3	0.90	2.51E-03	9.63E-02
ENSG00000010017	10048	RANBP9	-0.43	2.54E-03	9.69E-02
ENSG00000189056	5649	RELN	1.38	2.54E-03	9.69E-02
ENSG00000213988	7643	ZNF90	0.83	2.57E-03	9.74E-02
ENSG00000232810	7124	TNF	1.30	2.57E-03	9.74E-02
ENSG00000011295	54902	TTC19	-0.30	2.58E-03	9.75E-02
ENSG00000126062	11070	TMEM115	-0.34	2.61E-03	9.82E-02
ENSG00000211933	NA	IGHV6-1	1.44	2.61E-03	9.82E-02
ENSG00000181588	399664	MEX3D	-0.62	2.62E-03	9.84E-02
ENSG00000122188	54900	LAX1	1.37	2.64E-03	9.89E-02
ENSG00000162772	467	ATF3	-1.35	2.65E-03	9.90E-02
ENSG00000157064	23057	NMNAT2	1.33	2.67E-03	9.95E-02

UIP (n=12 samples); Non UIP (n=4 samples). Positive log2 fold change value indicates over-expression in UIP relative to Non UIP; negative log2 value indicates under-expression in UIP relative to Non UIP. In this analysis only patients with a smoking history were evaluated, hence this subset harbored only smokers.

[0187] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent application, foreign patents, foreign patent application and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, application and publications to provide yet further embodiments.

[0188] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

[0189] Some embodiments described herein relate to a computer storage product with a non-transitory computer-readable medium (also can be referred to as a non-transitory processor-readable medium) having instructions or computer code thereon for performing various computer-implemented operations. The computer-readable medium (or processor-readable medium) is non-transitory in the sense that it does not include transitory propagating signals per se (e.g., a propagating electromagnetic wave carrying information on a transmission medium such as space or a cable). The media and computer code (also can be referred to as code) may be those designed and constructed for the specific purpose or purposes. Examples of non-transitory computer-readable media include, but are not limited to, magnetic storage media such as hard disks, floppy disks, and magnetic tape; optical storage media such as Compact Disc/Digital Video Discs (CD/DVDs), Compact Disc-Read Only Memories (CD-ROMs), and holographic devices; magneto-optical storage media such as optical disks; carrier wave signal processing modules; and hardware devices that are specially configured to store and execute program code, such as Application-Specific Integrated Circuits (ASICs), Programmable Logic Devices (PLDs), Read-Only Memory (ROM) and Random-Access Memory (RAM) devices. Other embodiments described herein relate to a computer program product, which can include, for example, the instructions and/or computer code discussed herein.

[0190] Some embodiments and/or methods described herein can be performed by software (executed on hardware), hardware, or a combination thereof. Hardware modules may include, for example, a general-purpose processor, a field programmable gate array (FPGA), and/or an application specific integrated circuit (ASIC). Software modules (executed on hardware) can be expressed in a variety of software languages (e.g., computer code), including C, C++, Java™, Ruby, Visual Basic™, R, and/or other object-oriented, procedural, statistical, or other programming language and development tools. Examples of computer code include, but are not limited to, micro-code or micro-instructions, machine instructions, such as produced by a compiler, code used to produce a web service, and files containing

higher-level instructions that are executed by a computer using an interpreter. For example, embodiments may be implemented using imperative programming languages (e.g., C, Fortran, etc.), functional programming languages (e.g., Haskell, Erlang, etc.), logical programming languages (e.g., Prolog), object-oriented programming languages (e.g., Java, C++, etc.), statistical programming languages and/or environments (e.g., R, etc.) or other suitable programming languages and/or development tools. Additional examples of computer code include, but are not limited to, control signals, encrypted code, and compressed code.

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CLAIMS

What is claimed is:

1. A method of detecting whether a lung tissue sample is positive for usual interstitial pneumonia (UIP) or non-usual interstitial pneumonia (non-UIP), comprising:

Assaying the expression level of each of a first group of transcripts and a second group of transcripts in a test sample of a subject, wherein the first group of transcripts includes one or more sequence corresponding to any one of the genes overexpressed in UIP and listed in any of Tables 5, 7, 9, 10, 11, and 12 and the second group of transcripts includes one or more sequence corresponding to any one of the genes under-expressed in UIP and listed in any of Tables 5, 8, 9, 10, 11, or 12; and

comparing the expression level of each of the first group of transcripts and the second group of transcripts with reference expression levels of the corresponding transcripts to (1) classify said lung tissue as usual interstitial pneumonia (UIP) if there is (a) an increase in an expression level corresponding to the first group and/or (b) a decrease in an expression level corresponding to the second group as compared to the reference expression levels, or (2) classify the lung tissue as non-usual interstitial pneumonia (non-UIP) if there is (c) an increase in the expression level corresponding to the second group and/or (d) a decrease in the expression level corresponding to the first group as compared to the reference expression levels.

2. A method of detecting whether a lung tissue sample is positive for usual interstitial pneumonia (UIP) or non-usual interstitial pneumonia (non-UIP), comprising:

assaying by sequencing, array hybridization, or nucleic acid amplification the expression level of each of a first group of transcripts and a second group of transcripts in a test sample from a lung tissue of a subject, wherein the first group of transcripts includes one or more sequence corresponding to any one of the genes overexpressed in UIP and listed in any of Tables 5, 7, 9, 10, 11, and 12 and the second group of transcripts includes one or more sequence corresponding to any one of the genes under-expressed in UIP and listed in any of Tables 5, 8, 9, 10, 11, or 12; and

comparing the expression level of each of the first group of transcripts and the second group of transcripts with reference expression levels of the corresponding transcripts to (1) classify said lung tissue as usual interstitial pneumonia (UIP) if there is (a) an increase in an expression level corresponding to the first group and/or (b) a decrease in an expression level corresponding to the second group as compared to the reference expression levels, or (2) classify the lung tissue as non-usual interstitial pneumonia (non-UIP) if there is (c) an increase in the expression level corresponding to the second group and/or (d) a decrease in the expression level corresponding to the first group as compared to the reference expression levels.

3. A method of detecting whether a lung tissue sample is positive for UIP or non-UIP, comprising:

measuring the expression level of two or more transcripts expressed in the sample; and

using a computer generated classifier to classify the sample as UIP and non-UIP;

wherein the classifier was trained using a heterogeneous spectrum of non-UIP pathology subtypes comprising HP, NSIP, sarcoidosis, RB, bronchiolitis, and organizing pneumonia (OP).

4. The method of any one of claims 1-3, wherein the test sample is a biopsy sample or a bronchoalveolar lavage sample.

5. The method of any one of claims 1-3, wherein the test sample is fresh-frozen or fixed.

6. The method of any one of claims 1-3, wherein the expression levels are determined by RT-PCR, DNA microarray hybridization, RNASeq, or a combination thereof.

7. The method of any one of claims 1-3, wherein the method comprises detecting cDNA produced from RNA expressed in the test sample.

8. The method of claim 7, wherein prior to the detecting step, the cDNA is amplified from a plurality of cDNA transcripts.

9. The method of any one of claims 1-3, wherein one or more of the transcripts is labeled.

10. The method of any one of claims 1-3, further comprising measuring the expression level of at least one control nucleic acid in the test sample.

11. The method of any one of claims 1-3, wherein the lung tissue is classified as any one of interstitial lung diseases (ILD), a particular type of ILD, a non-ILD, or non-diagnostic.

12. The method of any one of claims 1-3, wherein the lung tissue is classified as either idiopathic pulmonary fibrosis (IPF) or Nonspecific interstitial pneumonia (NSIP).

13. The method of claim 1 or 2, wherein the method comprises assaying the test sample for the expression level of one or more transcripts of any one of SEQ ID NOS: 1-22.

14. The method of claim 13, further comprising assaying the test sample for the expression level of from 1 to 20 other genes.

15. The method of claim 3, wherein the method comprises assaying the test sample for the expression level of one or more transcripts of any one of SEQ ID NOS: 1-22.

16. The method of any one of claims 1-2, further comprising using smoking status as a covariate to the classification step of (1) or (2).

17. The method of claim 16, wherein smoking status is determined by detecting an expression profile indicative of the subject's smoker status.

18. The method of any one of the preceding claims, wherein classification of the sample comprises detection of the expression levels of one or more transcripts that are susceptible to smoker status bias, and wherein the transcripts that are susceptible to smoker status bias are weighted differently than transcripts that are not susceptible to smoker bias.

19. The method of any one of the preceding claims, wherein classification of the sample comprises detection of the expression levels of one or more transcripts that are susceptible to smoker status bias, and wherein the transcripts that are susceptible to smoker status bias are excluded from the classification step.

20. The method of any one of claims 1-2, wherein the first group comprises 2 or more different transcripts, or 3 or more, 4 or more, 5 or more, 10 or more, 15 or more, 20 or more, or more than 20 different transcripts.

21. The method of any one of claims 1-2, wherein the second group comprises 2 or more different transcripts, or 3 or more, 4 or more, 5 or more, 10 or more, 15 or more, 20 or more, or more than 20 different transcripts.

22. The method of claim 13 or 15, comprising detecting 2 or more different transcripts of any one of SEQ ID NOS:1-22, or 3 or more, 4 or more, 5 or more, 10 or more, 15 or more, 20 or more, or more than 20 different transcripts of any one of SEQ ID NOS:1-22.

23. The method of claim 13 or 15, comprising assaying the test sample for the expression level of all of the transcripts of SEQ ID NOS: 1-22.

24. The method of claim 15, 22, or 23, further comprising assaying the test sample for the expression level of from 1 to 20 other genes.

25. The method of claim 24, wherein the other genes comprise or consist of HMCN2, ADAMTSL1, CD79B, KEL, KLHL14, MPP2, NMNAT2, PLXDC1, CAPN9, TALDO1, PLK4, IGHV3-72, IGKV1-9, and CNTN4.

26. The method of claim 3, further comprising using smoking status as a covariate to the classification step.

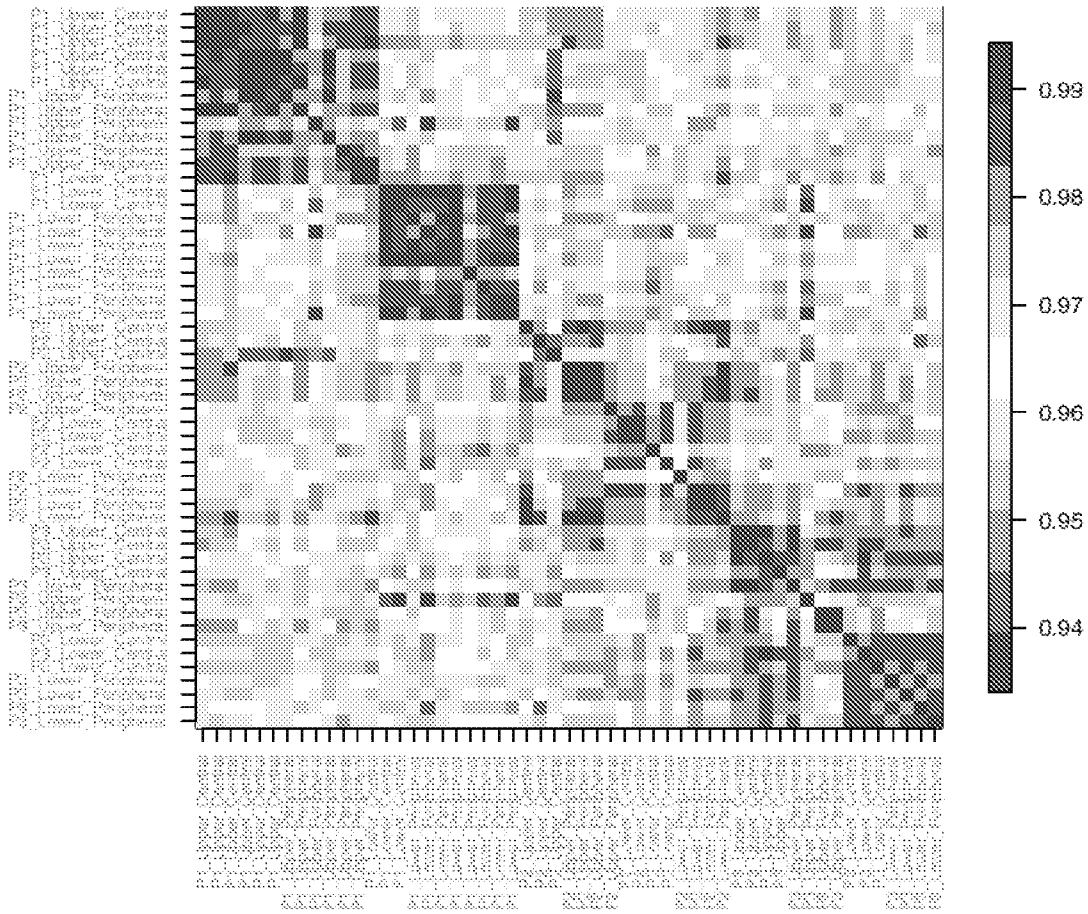
27. The method of claim 16 or 27, wherein the method uses smoking status as a covariate prior to the classification step.

28. The method of any one of the preceding claims, comprising implementing a classifier trained using one or more feature selected from gene expression, variants, mutations, fusions, loss of heterozygoxity (LOH), and biological pathway effect.

29. The method of claim 29, wherein the classifier is trained using features including gene expression, sequence variants, mutations, fusions, loss of heterozygoxity (LOH), and biological pathway effect.

30. The method of any one of the preceding claims, wherein the classification step further comprises detecting sequence variants in the test sample and comparing the sequence variants to the respective sequences in a reference sample to classify the sample as UIP or non-UIP.

FIG. 1



FIGS. 2A-2D

FIG. 2A

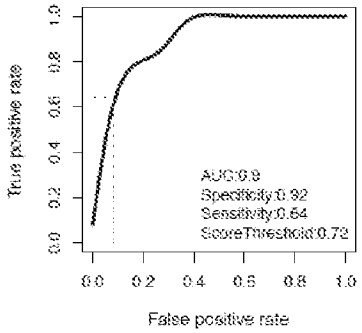


FIG. 2B

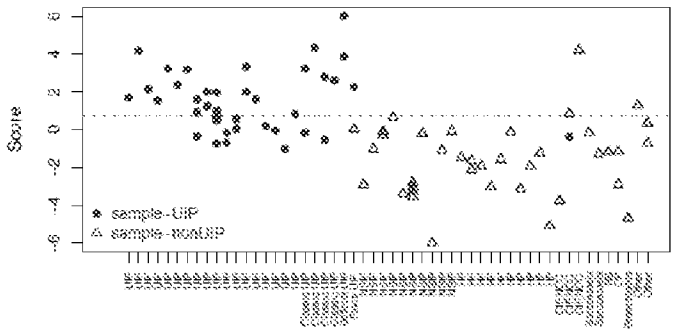


FIG. 2C

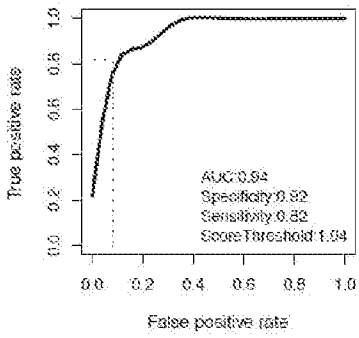
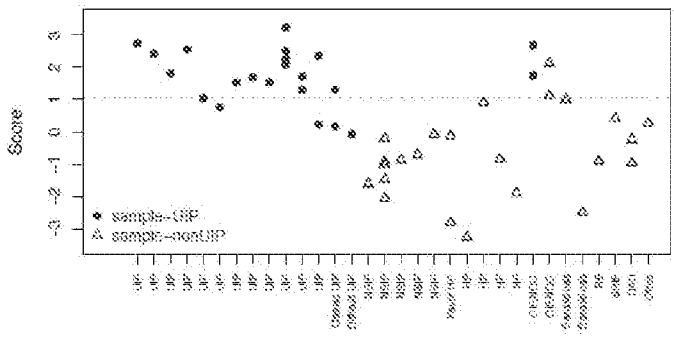


FIG. 2D



FIGS. 3A-3D

FIG. 3A

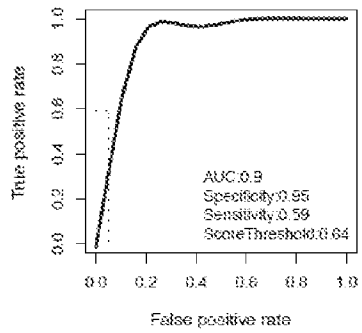


FIG. 3B

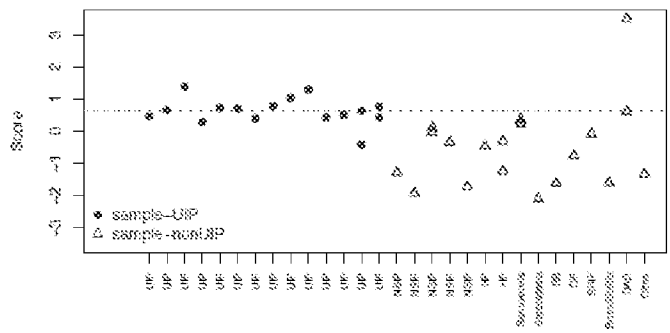


FIG. 3C

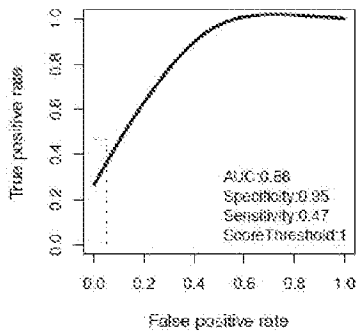


FIG. 3D

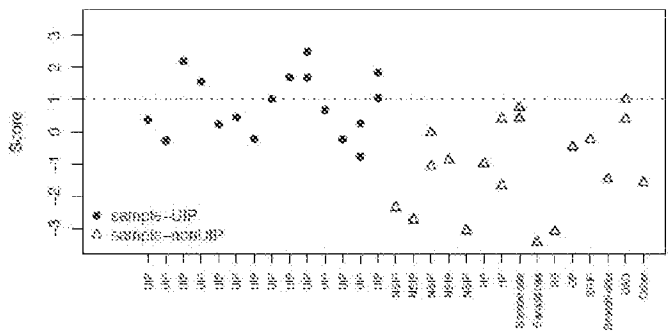


FIG. 4

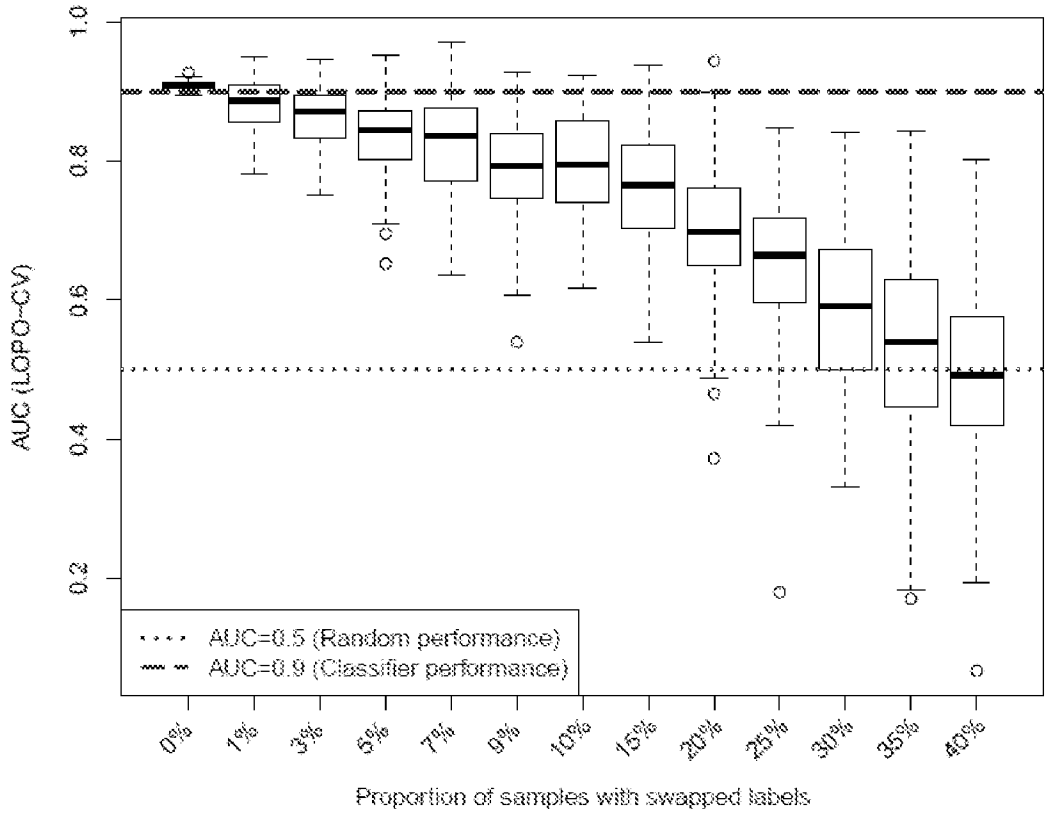

AUC (LOPO-CV)
1.0
0.8
0.6
0.4
0.2
AUC=0.5 (Random performance)
AUC=0.9 (Classifier performance)
0%
1%
3%
5%
7%
9%
10%
15%
20%
25%
30%
35%
40%
Proportion of samples with swapped labels

FIG.5

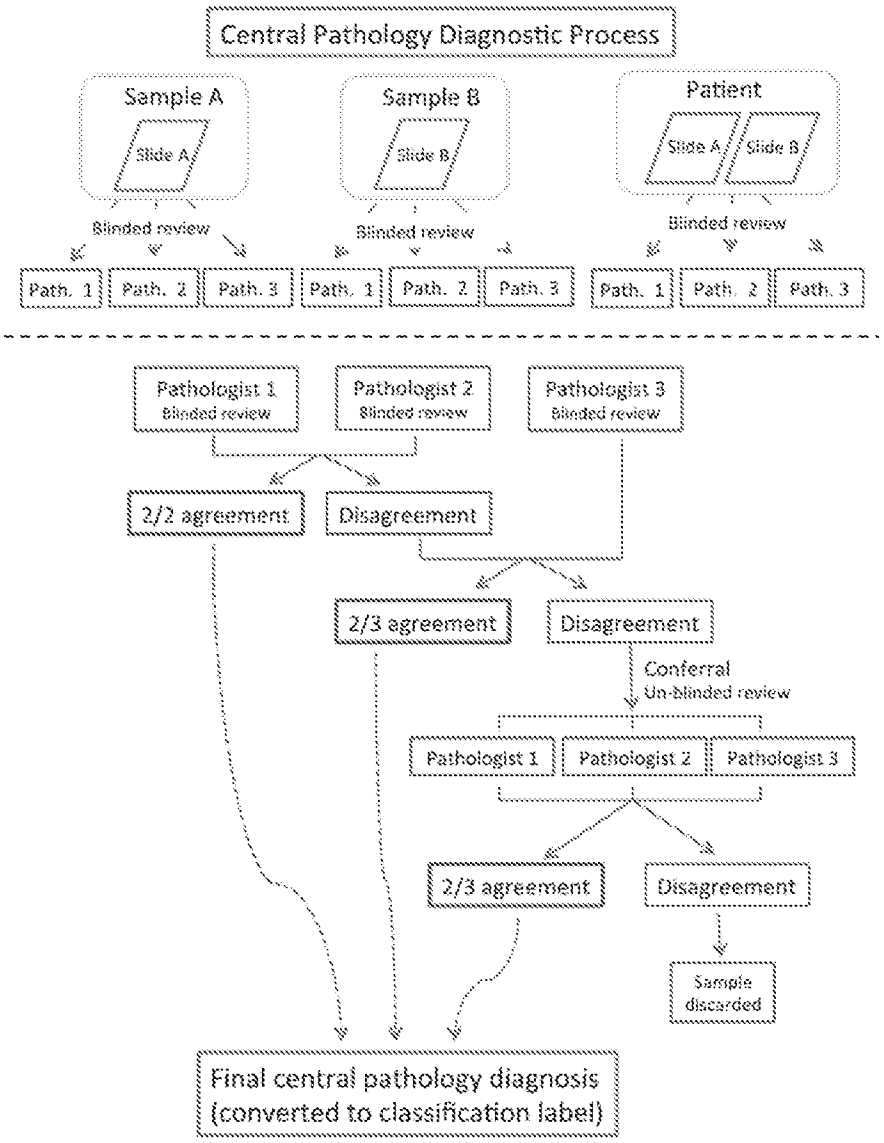

Central Pathology Diagnostic Process
Sample A
Slide A
Sample B
Slide B
Patient
Slide A
Slide B
Blinded review
Blinded review
Blinded review
Path. 1
Path. 2
Path. 3
Path. 1
Path. 2
Path. 3
Path. 1
Path. 2
Path. 3
Pathologist 1
Blinded review
Pathologist 2
Blinded review
Pathologist 3
Blinded review
2/2 agreement
Disagreement
2/3 agreement
Disagreement
Conferral
Un-blinded review
Pathologist 1
Pathologist 2
Pathologist 3
2/3 agreement
Disagreement
Sample discarded
Final central pathology diagnosis (converted to classification label)

FIG. 6

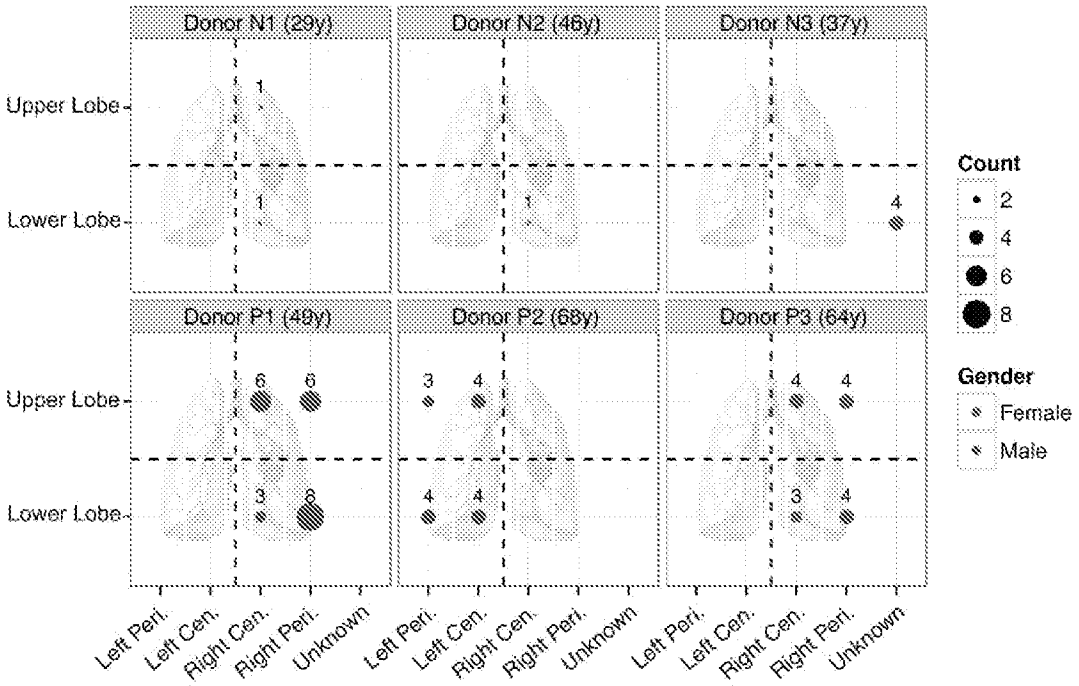

Donor N1 (29y)
Donor N2 (46y)
Donor N3 (37y)
Donor P1 (49y)
Donor P2 (68y)
Donor P3 (64y)
Upper Lobe
Lower Lobe
Left Peri.
Left Cen.
Right Cen.
Right Peri.
Unknown
Count
2
4
6
8
Gender
Female
Male

FIG. 7A

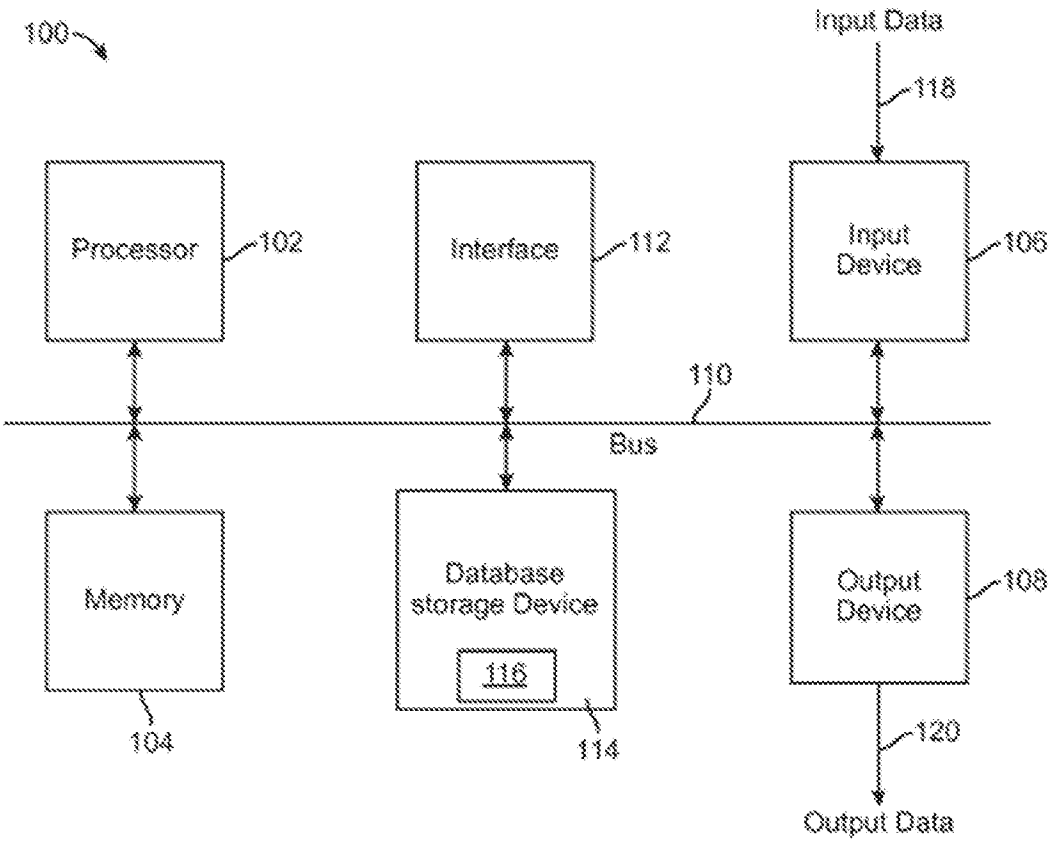

100
Input Data
118
Processor
102
Interface
112
Input Device
106
110
Bus
Memory
104
Database storage Device
116
114
Output Device
108
120
Output Data

FIG. 7B

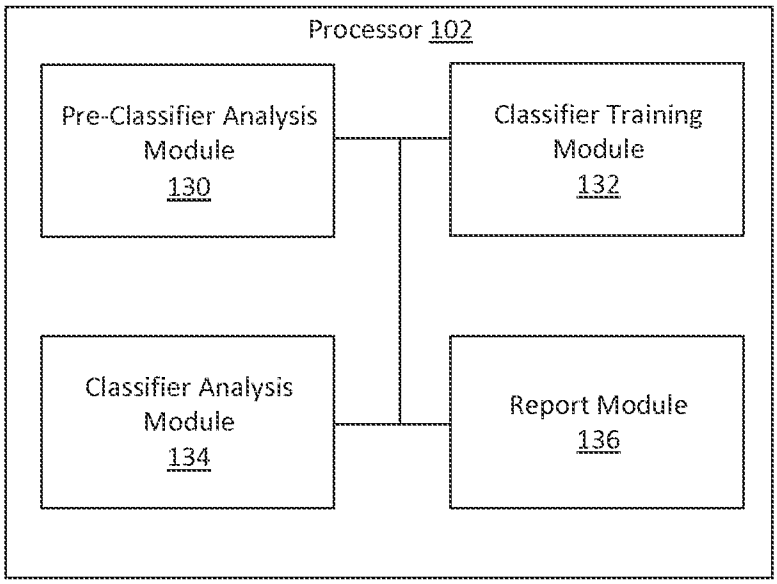

Processor 102
Pre-Classifier Analysis Module 130
Classifier Training Module 132
Classifier Analysis Module 134
Report Module 136

FIG. 7C

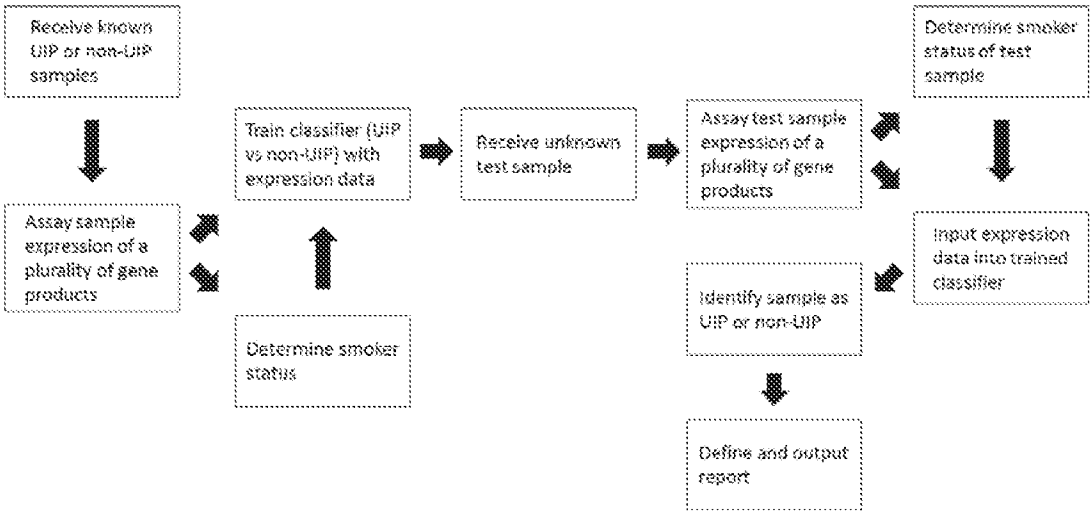
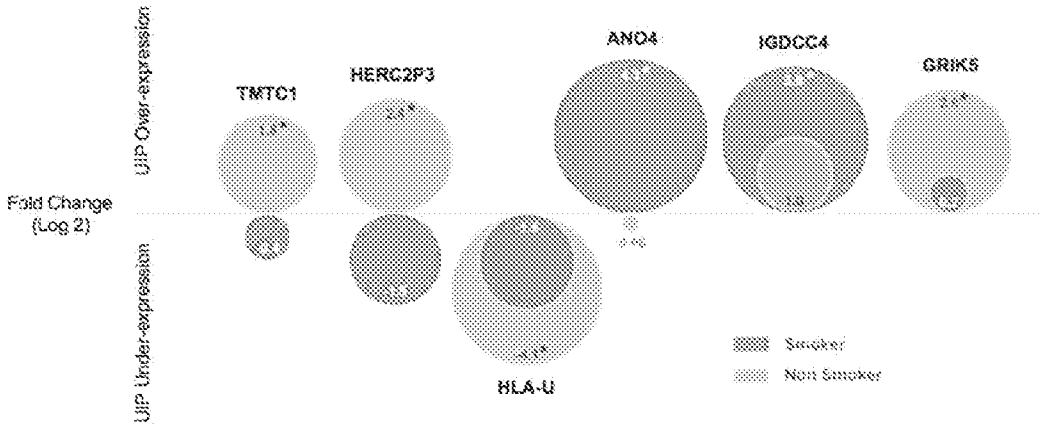

Receive known UIP or non-UIP samples
Assay sample expression of a plurality of gene products
Determine smoker status
Train classifier (UIP vs non-UIP) with expression data
Receive unknown test sample
Assay test sample expression of a plurality of gene products
Input expression data into trained classifier
Determine smoker status of test sample
Identify sample as UIP or non-UIP
Define and output report

FIG. 8

DE Genes	All Combined UIP vs. Non UIP	Smokers Only UIP vs. Non UIP	Non Smokers Only UIP vs. Non UIP
FDR p value <0.01	26	64	671

FIG. 9



FIGS. 10A-10D

FIG. 10A

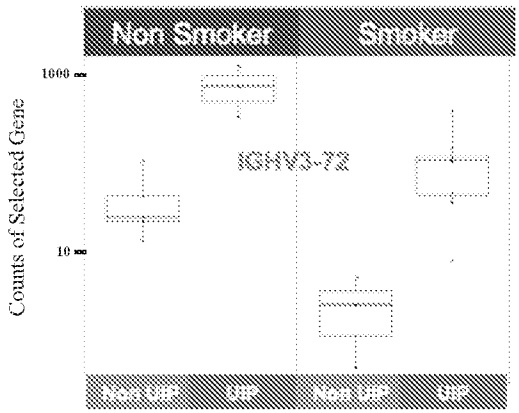


FIG. 10B

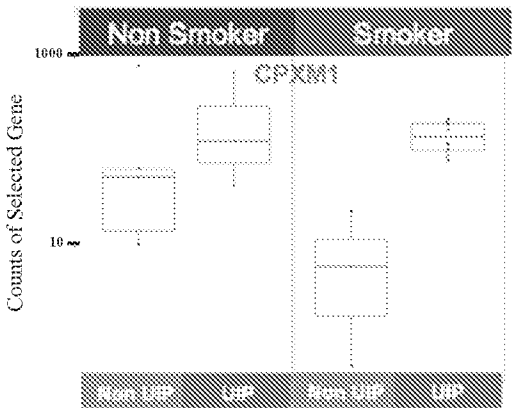


FIG. 10C

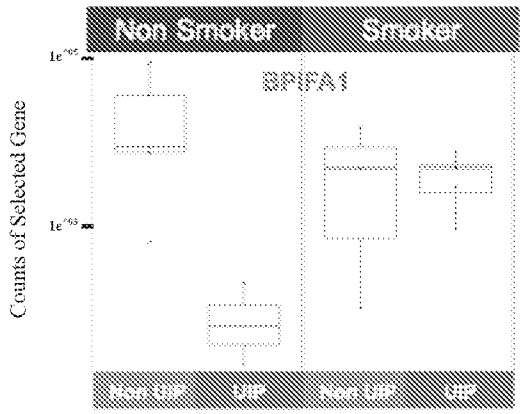
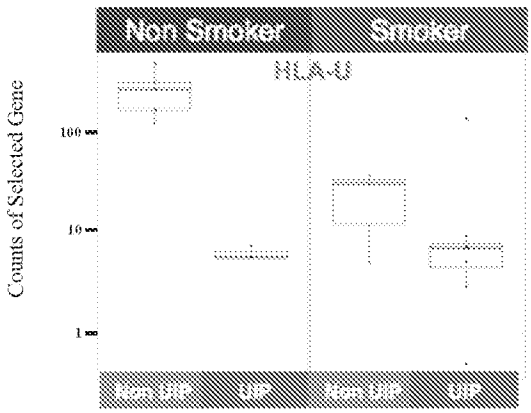


FIG. 10D



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/59309

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00, 39/395; A61P 11/00 (2016.01)
CPC - A61K 38/00, 39/3955, 45/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 38/00, 39/395; A61P 11/00 (2016.01)
CPC: A61K 38/00, 39/3955, 45/06, 2039/505

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO Discovery; IP.com; Google; Google Scholar; Google Patents
KEYWORDS: lung, tissue, UIP, non-uip, expression, level, transcript, reference

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	WO 2014/1544564 A2 (VERACYTE INC.) September 18, 2014; abstract; paragraphs [003], [0010], [0012], [0015], [0016], [0041], [0042], [0050], [0067], [0071], [0073], [0078], [0087], [0098], [00146], [00148]- [00150], [00161], [00239], [00240], [00279], [00297]	1-3, 4/1-3, 5/1-3, 6/1-3, 7/1-3, 8/7/1-3, 9/1-3, 10/1-3, 11/1-23, 12/1-3, 16/1-2, 17/16/1-2, 20/1-2, 21/1-2, 26 --- 13/1-2, 14/13/1-2, 15
Y	US 2014/0179771 A1 (MODERNA THERAPEUTICS, INC.) June 26, 2014; paragraph [0095], [0099], [0784]	13/1-2, 14/13/1-2, 15
A	US 2013/0029873 A1 (DE PERROT, M et al.) January 31, 2013; entire document	1-17, 20, 21, 26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 February 2016 (29.02.2016)	11 MAR 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/59309

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18, 19, 22-25, 27-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-17, 20, 21, 26, markers HLA-F, CDKL2, SEQ ID NOs: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.