

STEROID RESPONSIVE NUCLEIC ACID EXPRESSION AND PREDICTION OF DISEASE ACTIVITY

RELATED APPLICATION

[0001] This application claims priority to US Patent Application Number 60/790,474, filed 7 April 2006, which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

[0002] The invention relates to methods for detecting nucleic acid and protein expression modulated by steroids and using steroid responsiveness of a subject in predicting and monitoring disease activity.

BACKGROUND OF THE INVENTION

[0003] Steroids are used to ameliorate disease activity associated with immune disorders such as graft rejection, systemic lupus erythematosus (SLE), multiple sclerosis (MS) and cytomegalovirus (CMV) infection. Although steroids are used clinically to treat hyperactivity of the immune system, prolonged treatment has deleterious effects including diabetes, osteoporosis and weight gain. Given these and other side effects, clinicians avoid prescribing high dosages of steroid any longer than necessary. Since flare of immune disorders and transplantation require the use of steroids as an ongoing treatment, it is desirable to determine the steroid responsiveness of a subject in order to optimize outcome. An essential component of providing effective immunosuppression is monitoring subject or transplant status. In transplant patients, this monitoring is organ, tissue or cell-specific. For example, monitoring a subject with a cardiac transplant involves taking a biopsy of heart muscle and having a pathologist examine it for cytological evidence of rejection. Such biopsies are expensive, invasive, and painful and interpretation can only be focused on the biopsied cells, not the whole organ.

[0004] Although glucocorticoid induction of genes correlated with immune response has been studied *in vitro* (Galon et al. (2002) FASEB Journal 16:61-71); there is a need for methods to detect *in vivo* expression of steroid modulated nucleic acids. The present invention addresses this need by diagnosing and monitoring steroid responsiveness or immunological status, predicting flares or graft rejection, and designing, evaluating or monitoring treatment efficacy.

SUMMARY OF THE INVENTION

[0005] The present invention provides methods for detecting *in vivo* expression of nucleic acids and proteins modulated by steroid administration and metabolism. The invention presents a method of diagnosing or monitoring steroid responsiveness of a subject comprising detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression is correlated with steroid administration or dosage and applying at least one statistical method to the expression of the diagnostic set to diagnose or monitor steroid responsiveness of the subject.

[0006] In one embodiment, the diagnostic set further comprises at least one steroid modulated nucleic acid selected from each of at least two of the clusters of Table 1. In a second embodiment, the diagnostic set further comprises two or more steroid modulated nucleic acids selected from Table 2. In a third embodiment, the diagnostic set further comprises two or more steroid modulated nucleic acids selected from Table 3. In one aspect, detecting the expression of the diagnostic set of steroid modulated nucleic acids further comprises using hybridization or quantitative real-time polymerase chain reaction (RT-PCR) and a sample obtained from the subject by any sampling means. In a second aspect, the sample is a blood sample, and RNA is isolated from the peripheral blood mononuclear cells (PMBC) of the blood sample. In a third aspect, the statistical method is K-means clustering that produces clusters of genes that are correlated by p-value and their expression in a cell type or pathway or a prediction algorithm selected from a linear algorithm, a logistic regression algorithm, and a voting algorithm that produces a single value or score.

[0007] In a fourth embodiment, the diagnostic set further comprises selecting at least two oligonucleotides or a probe set to detect the expression of each steroid modulated nucleic acid of the diagnostic set. The invention also presents a kit comprising the oligonucleotides or probe sets that detect the expression of each steroid modulated nucleic acid of the diagnostic set. The invention further presents a method for diagnosing or monitoring steroid responsiveness of a subject comprising detecting the expression of nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3.

[0008] The invention additionally presents a method for predicting rejection or non-rejection in a subject with a transplant comprising detecting expression of a diagnostic set of at least two

steroid modulated nucleic acids in a sample from the subject wherein the expression of the steroid modulated nucleic acids correlates with transplant rejection or non-rejection, and applying at least one statistical method to the expression of the diagnostic set of steroid modulated nucleic acids to predict rejection or non-rejection.

[0009] In one embodiment, the diagnostic set of steroid modulated nucleic acids further comprises two or more nucleic acids selected from Tables 1-3. In one aspect, detecting the expression of the diagnostic set of steroid modulated nucleic acids further comprises using RT-PCR and RNA isolated from PMBCs. In a third aspect, the statistical method is a prediction algorithm selected from a linear algorithm, a logistic regression algorithm, and a voting algorithm that produces a single value or score that correlates with rejection or non-rejection. In a fourth aspect, the score that correlates with non-rejection is ≤ 20 and the score that correlates with rejection is ≥ 30 . The invention yet further presents a method of predicting rejection or non-rejection comprising detecting the expression of a diagnostic set of steroid modulated nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3.

[0010] The invention further presents a method of diagnosing or monitoring the status of a subject with a transplant comprising detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression is correlated with dysfunction or rejection of the transplant, and applying at least one statistical method to the expression of the nucleic acids to monitor the status of the transplant. In one embodiment, the diagnostic set further comprises two or more nucleic acids selected from Tables 1-3. In a second embodiment, RT-PCR is used with RNA isolated from PMBC to detect expression of the steroid modulated nucleic acids and the expression is analyzed using a prediction algorithm that produces single value or score that correlates with the status of the subject with the transplant. In a third embodiment, diagnosing and monitoring the status of a subject with a transplant further comprises detecting the expression of a diagnostic set of steroid modulated nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3.

[0011] The invention also presents method for designing and monitoring a treatment plan for a subject with a transplant or an immune disorder comprising detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression correlates with the steroid responsiveness of the subject, and using the

expression of the diagnostic set of steroid modulated nucleic acids to design and monitor the treatment plan of the subject. In one embodiment, the diagnostic set of steroid modulated nucleic acids comprises two or more nucleic acids selected from Tables 1-3. In a second embodiment, RT-PCR is used with RNA isolated from PMBC to detect expression of the steroid modulated nucleic acids and the expression is analyzed using a prediction algorithm that produces single value or score that correlates with the steroid responsiveness of the subject. In a third embodiment, diagnosing and monitoring the status of a subject with a transplant or immune disorder further comprises detecting the expression of a diagnostic set of steroid modulated nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3 whose expression correlates with steroid responsiveness of a subject. In one aspect, the transplant is selected from bone marrow, heart, kidney, liver, lung, pancreas, pancreatic islets, stem cells, xenotransplants, and artificial implants. In another aspect, the immune disorder is selected from cytomegalovirus infection, multiple sclerosis, and systemic lupus erythematosus.

[0012] The invention yet still further presents a method for using primers and probe sets to detect steroid responsiveness of a subject with a transplant or an immune disorder comprising designing and generating primers or probe sets for nucleic acids whose expression is modulated by steroid administration or dosage, and using RT-PCR and the primers or probe sets on a sample from the subject to detect steroid responsiveness. In one embodiment, the nucleic acids whose expression is modulated by steroid administration or dosage are selected from Tables 1-3. In a second embodiment, the primers and probe sets are used in a diagnostic kit.

BRIEF DESCRIPTION OF THE TABLES

[0013] Table 1 presents ten clusters of genes whose nucleic acid and protein expression is modulated by steroids. Column 1 shows cluster number; column 2, microarray probe ID from Human Genome CGH 44A Microarray (Agilent Technologies); column 3, gene symbol; column 4, average p-value for expression of the nucleic acid in CARGO and LARGO; column 5, average Pearson correlation for expression of the nucleic acid in CARGO and LARGO; column 6, p-value for the expression of the nucleic acid in CARGO, column 7, p-value for the expression of the nucleic acid in LARGO; and column 8, the name of the gene as it appears in the GenBank database (NCBI, Bethesda MD).

[0014] Table 2 summarizes steroid modulated nucleic acid expression for 104 subject post-transplant samples and a subset of 74 samples ≤ 180 days post-transplant. Column 1 shows the nucleic acids whose probe sets were used in RT-PCR to detect expression in post-transplant subject samples. The overall score refers to the single value produced from all scores using a linear discriminant algorithm. Columns 2-5 show the data for rejection (R) subjects, non-rejection (NR) subjects, the ratio, and p-values for all days post-transplant (index), respectively. Columns 6-9 show the data for rejection (R) subjects, non-rejection (NR) subjects, the ratio, and p-values for <180 days post transplant samples (subset), respectively. Significant p-values are shown in red typeface.

[0015] Table 3 presents RT-PCR data for 33 nucleic acids expressed in pathways having genes modulated by steroids or regulating T-cell homeostasis. Column 1 of Table 3 shows the gene symbol; columns 2 and 3, the fold change and p-value for R (n=38)/NR (n=55) at all times post-transplant; columns 4 and 5, the fold change and p-value for R (n=27)/NR (n=40) at ≤ 180 days post-transplant; and column 6, the gene name.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The present invention addresses needs in the art by providing methods for detecting the *in vivo* expression of nucleic acids modulated by steroid administration or metabolism. The invention also provides methods for diagnosing and monitoring steroid responsiveness of a subject by detecting the expression of nucleic acids modulated by steroids. The invention uses detection of nucleic acids modulated by steroids to predict disease activity or transplant non-rejection or rejection and to determine status of an immune disorder or transplant. Such methods can be used to fine-tune immunosuppressant therapy and, more importantly, to reduce the number of invasive and costly tests and procedures that a subject must undergo. In particular, the invention can be used to predict transplant non-rejection or rejection. For example the invention can be used to predict transplant non-rejection or rejection allowing a clinician to reduce the number of biopsies performed in the first 180 days post-transplant or to begin anti-rejection therapy before cytological evidence of rejection is detectable. The invention also provides methods for evaluating the need for post-transplant monitoring and treatment or determining a subject's near-term prognosis based on steroid modulated nucleic acid expression.

Definitions

[0017] Unless defined otherwise, all scientific and technical terms are understood to have the same meaning as commonly used in the art to which they pertain. For the purpose of the present invention, the following terms are defined.

[0018] “Amplification” refers to any device, method or technique that can make copies of a nucleic acid. It can be achieved using a thermal cycler or a thermal gradient device and a polymerase chain reaction (PCR) technique such as linear amplification (cf. USPN 6,132,997), rolling circle amplification, and the like. Further, amplification and detection can be combined as in Real-Time PCR (RT-PCR) using TAQMAN protocols and the Prism 7900HT Sequence Detection system and software (Applied Biosystems (ABI), Foster City CA).

[0019] “Array” refers to an ordered arrangement of at least two samples -- nucleic acids, proteins or antibodies -- in solution or on a substrate where at least one of the samples represents a control and/or normal sample and the other, a sample of diagnostic or prognostic interest. The ordered arrangement ensures that the size and signal intensity of each labeled complex, formed between at least one reagent and at least one sample to which the reagent specifically binds is readily detectable.

[0020] “Clusters” refers to groups of nucleic acids with expression that is directly or indirectly regulated by and correlated with the administration or metabolism of a steroid.

[0021] “Diagnostic set” refers to at least two nucleic acids whose expression is modulated by steroids and whose nucleic acids, oligonucleotides, primers and probe sets can be used in nucleic acid technologies or encoded proteins and antibodies or affinity reagents thereto can be used in protein technologies.

[0022] “Expression” refers to differential expression--increased or decreased expression as detected by presence, absence, or change in the amount of nucleic acid or protein expressed in a sample -- as presented in a gene expression profile. A “gene expression profile” refers to the identification, characterization, quantification, and representation of a plurality of nucleic acids expressed in a sample from a subject as measured using nucleic acid or protein technologies. Nucleic acid expression is detected using nucleic acid technologies and mature mRNA transcript and/or regulatory sequences such as promoters, enhancers, introns, mRNA-processing intermediates, and 3' untranslated regions. A gene expression profile from a subject can be

compared with reference gene expression profiles based on detection of nucleic acid expression in control or normal, diseased, or treated samples.

[0023] “Immune disorders” refers to conditions, disorders and diseases associated with immunological response including but not limited to acute respiratory distress syndrome, Addison’s disease, allograft rejection, ankylosing spondylitis, Takayasu’s arteritis, arteriosclerosis, asthma, atherosclerosis, congestive heart failure, primary sclerosing cholangitis, Churg-Strauss syndrome, CREST syndrome, Crohn’s disease, ulcerative colitis, diabetes mellitus, emphysema, glomerulonephritis, Wegener’s granulomatosis, Grave’s disease, autoimmune hepatitis, Kawasaki’s syndrome, systemic lupus erythematosus, multiple sclerosis, myasthenia gravis, myelofibrosis, pancreatitis, polyarteritis nodosa, polymyositis, psoriasis, Raynaud’s disease, Reiter’s syndrome, rheumatoid arthritis, scleroderma, primary biliary sclerosis, systemic sclerosis, sepsis, septic shock syndrome, Sjogren’s disease, ankylosing spondylitis, primary thrombocythemia, Hashimoto’s thyroiditis, systemic vasculitis, Whipple’s disease, complications of cancer, viral infection including CMV infection, bacterial infection, fungal infection, parasitic infection, protozoal infection, helminthic infection, and trauma.

[0024] “Immunosuppressant” refers to any therapeutic agent that suppresses immune response in a subject such as anticoagulents, antimalarials, heart drugs, non-steroidal anti-inflammatory drugs, and steroids including but not limited to aspirin, azathioprine, chloroquine, corticosteroids, cyclophosphamide, cyclosporin A, dehydroepiandrosterone, deoxyspergualin, dexamethasone, everolimus, fenoprofen, hydralazine, hydroxychloroquine, immunoglobulin, ibuprofen, indomethacin, leflunomide, ketoprofen, meclorphenamate, mepacrine, 6-mercaptopurine, methotrexate, mizoribine, mycophenolate mofetil, naproxen, prednisone, methylprednisone, rapamycin (sirolimus), solumedrol, tacrolimus (FK506), thymoglobulin, tolmetin, tresperimus, triamcinoline, and the like.

[0025] “Monitoring” refers to repetitive testing for and detection of nucleic acid expression that provides useful information about a subject’s health or disease status. Monitoring can include determining prognosis, risk-stratification, and efficacy of a particular drug; detecting subject response to a drug or ongoing therapy; predicting susceptibility, rejection or non-rejection, or disease activity; diagnosing onset, flare or complication of a disease; following disease progression or providing information related to a subject’s status over time; selecting subjects most likely to benefit from a particular drug or experimental therapy especially where administration of that drug works for a small subset of subjects or where the drug does not have a

label for a particular immune disorder; and screening a subject population to decide to use a more or less invasive or costly test; for example, moving from a non-invasive blood test to a more invasive option such as biopsy.

[0026] “Nucleic acid technology” refers to any and all devices, methods and systems used to detect expression of nucleic acids and produce a gene expression profile including but not limited to methods using arrays for hybridization, amplification in PCR, quantitative RT-PCR, TAQMAN protocol RT-PCR, multiplex PCR, thermal gradient devices, and the like, or hybridization in solution or on a substrate containing cDNAs, genomic DNAs, locked nucleic acids (LNAs), oligonucleotides, primers, peptide nucleic acids, polynucleotides, probe sets, RNAs and the like.

[0027] “Prediction” or “predicting” refers to the use of gene expression profile to provide information about a subject’s health or the status of a disease, patient or transplant and can include determination of prognosis, risk-stratification, prediction of outcomes, and the like.

[0028] A “probe set” refers to groups of oligonucleotides or primers that can be used with a nucleic acid technology to detect groups of two or more nucleic acids. Primers in a probe set can contain rare or artificial nucleotides, be of any size useful in a nucleic acid technology, designed to detect a particular region or splice variant of a gene, labeled with one or more detectable moieties, and used in solution or attached to a substrate.

[0029] “Protein technology” refers to any and all devices, methods and systems that can be used to detect a peptide, polypeptide or protein expressed by a steroid modulated nucleic acid or gene and produce a gene expression profile including but not limited to activity assays, affinity assays, antibody or protein arrays, chromatographic separation, colorimetric assays, two-dimensional gel electrophoresis, ELISA, fluorescent-activated cell sorting, mass spectrophotometric detection, protein-fusion reporter constructs, western analysis, and the like. Protein expression, although time delayed, is correlated with and mirrors nucleic acid expression.

[0030] “Sample” is used in its broadest sense and refers to any biological material used for cytological or histological evaluation or to measure nucleic acid expression and obtained from a subject by any sampling means known to those of skill in the art. A sample can comprise a bodily fluid such as ascites, bile, blood, cerebrospinal fluid, synovial fluid, lymph, pus, semen, sputum, urine; the soluble fraction of a cell preparation, an aliquot of media in which cells were grown; a chromosome, an organelle, or membrane isolated or extracted from a cell; cDNA,

genomic DNA, or RNA including but not limited to hnRNA, mRNA, mRNA processing intermediates, rRNA, and tRNA in solution or bound to a substrate; a cell; a cell, tissue or organ biopsy, and the like. Preferred samples for diagnosis, prognosis, or monitoring of immunological status are leukocytes, peripheral blood mononuclear cells (PBMC), or serum derived from whole blood.

[0031] “Sampling means” refers to any instrumentation and protocols for obtaining a biological sample and includes but is not limited to aspiration of a body fluid, aspiration of fluid following lavage, a biopsy (bronchoscopy or endoscopy) of cells, a tissue or organ, drawing of central or peripheral blood, and the like.

[0032] A “statistical method” refers to methods including but not limited to analysis of variance, canonical analysis, classification algorithms, classification and regression trees, cluster analysis including K-means clustering, factor analysis, Fisher’s Exact test, k-nearest neighbor, linear algorithm, linear discriminatory analysis, linear regression, logistic algorithm, multidimensional scaling analysis, multiple regression, nearest shrunken centroids classifier, Pearson correlation, prediction algorithm, significance analysis of microarrays, one-tailed T-tests, two-tailed T-tests, voting algorithm, Wilcoxon’s signed ranks test, and the like.

[0033] “Status” refers to any and all aspects of immune response in a subject who has an immune disorder or transplant including deterioration, improvement, progression, remission, or stability as determined from analyzing one or more samples from that subject for nucleic acid or protein expression that correlates with the degree and nature of response, steroid treatment or related complications including autoimmune cellular destruction, acute rejection, chronic rejection, humoral rejection, vasculopathy, and the like.

[0034] “Steroid modulated” refers to any gene product, nucleic acid or protein, whose expression is correlated with and results directly or indirectly from the administration or metabolism of steroids. For example, genes that have a steroid dependent regulatory element (sdre) in their promoter region (Dillner and Sanders (2002) J Biol Chem 277:33890-33894) are steroid modulated, primary response genes regulated by the presence and/or dosage of steroids. These primary response genes are often transcription factors that activate one or more indirectly affected, secondary response genes or pathways.

[0035] “Steroid responsive” or “steroid responsiveness” refers to any aspect of the immunological response of a subject to the administration or metabolism of steroids including

improvement or worsening of symptoms, adjustment in dosage, change to another immunosuppressant, and the like.

[0036] “Subject” refers to an individual or patient who develops an infection, has an immune disorder or has received any allograft that elicits an immune response.

[0037] “Substrate” refers to any rigid or semi-rigid support to which antibodies, nucleic acids or proteins are bound and includes magnetic or nonmagnetic beads, capillaries or other tubing, chips, fibers, filters, gels, membranes, microparticles, plates, polymers, slides, and wafers with a variety of surface forms including channels, columns, pins, pores, trenches, wells and the like made from any natural or synthetic material or combination thereof.

[0038] “Transplant” refers to a subject’s own genetically modified cells, or tissues grown from those cells; cells, tissues or organs from another subject or from an animal of a different species; and artificial implants such as mechanical or partially mechanical replacement organs.

[0039] “Transplant rejection” as detected or predicted using the methods and materials of the present invention refers to the rejection of bone marrow, heart, kidney, liver, lung, pancreas, pancreatic islets, stem cells, xenotransplants, and artificial implants.

[0040] “Quiescence” refers to the absence of signs or symptoms of histological or immunological response. For example, a diagnosis of remission in a subject with an immune disorder or non-rejection in a transplant patient indicates successful repression of immunological response and/or treatment with an immunosuppressant.

Description of the Invention

[0041] The correlation between the administration of steroids and the differential expression of steroid modulated nucleic acids and proteins provides an opportunity for developing pharmacogenomic markers for diagnosing and monitoring subjects with transplants, immune disorders such as SLE or MS, and CMV infection. As described in the Examples, the present invention provides methods, diagnostic sets of steroid modulated nucleic acids selected from Tables 1-3, and reagents such as antibodies, affinity reagents, primers and probe sets that can be used for determining, diagnosing, evaluating, monitoring, or predicting disease activity, non-rejection, rejection, status of a transplant or of an immune disorder, steroid responsiveness, and treatment plan of a subject with a transplant or immune disorder. In one embodiment, the ability to predict acute rejection can be used to begin immediate anti-rejection therapy while the ability

to predict non-rejection can be used to determine the need for and timing of costly and invasive procedures such as biopsies. The invention additionally provides methods for designing and monitoring a treatment plan for a subject with an immune disorder or transplant and for evaluating the need for post-diagnosis or post-transplant monitoring and treatment.

[0042] The methods of the invention used RNA isolated from PBMC samples obtained from subjects enrolled in the Cardiac Allograft Rejection Gene Expression Observational (CARGO) and the Lung Allograft Rejection Gene Expression Observational (LARGO) studies. The samples were processed as described in Example 8 and used to study gene expression using nucleic acid technologies.

[0043] Microarray studies of gene expression were performed using the protocols described in Examples 9 and 10. These studies identified steroid modulated nucleic acids in the CARGO and LARGO samples from subjects treated with 1-100 mg doses of steroid as described in Example 1. Iterative cluster analysis and similarity testing as described in Example 4 were used to identify the nucleic acids modulated by steroids presented in Table 1. An exemplary RT-PCR study, carried out using the protocols described in Examples 13, used probe sets for 20 informative genes to investigate steroid responsiveness CARGO samples. The results of this study, as described in Example 5 and presented in Table 2, revealed that the expression of the nucleic acids known to be modulated by steroids were important both in diagnosing and monitoring steroid responsiveness and in predicting transplant rejection and non-rejection.

[0044] When the data from the exemplary RT-PCR study showed that differential expression of nucleic acids encoding FLT3, IL1R2, ITGAM, and PDCD1 proteins or fragments thereof was highly predictive of non-rejection within 180 days of transplant, a second study was performed to test additional nucleic acids in related pathways. Table 3 presents the results of the pathways RT-PCR study on 33 genes in the IL-1 or PDCD1 pathway, the ligand for FLT3, and genes induced and expressed in T cells. Using a p-value <0.05, the genes encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3 protein and fragments thereof showed differential expression correlated with rejection.

[0045] Primers or probes sets that detect expression of at least one nucleic acid from the steroid modulated genes of Tables 1-3 can be used in a diagnostic set to carry out the methods of the invention. In one embodiment, the steroid modulated nucleic acids of the invention were

used to design, select, and test primers and probe sets that can be used to detect steroid responsiveness in a sample from a subject as described in Examples 11 and 12. In another embodiment, antibodies or other affinity reagents specifically binding to a protein or a fragment thereof, expressed from steroid modulated genes of Tables 1-3, can be used in a diagnostic set to carry out the methods of the invention. Protein expression and antibody production and testing are described in Examples 15 and 16.

[0046] In a preferred embodiment, the methods and diagnostic sets of this invention can be used on clinically stable subjects, those showing no histological signs of rejection in endomyocardial biopsies (EMB) within 180 days of transplant to predict the probability that transplant rejection will occur within the subsequent 12 weeks. For example, a prediction algorithm was applied to the nucleic acid expression from exemplary RT-PCR studies to produce a single score for each subject. Then quartile analysis was applied to the single scores as described in Example 6. When used in longitudinal studies of ≤ 180 days post transplant, the score produced by the algorithm distinguished clinically stable cardiac transplant subjects who did not reject their transplant in the subsequent 12 weeks, 98.9% with a score ≤ 20 , from those who progressed to acute cellular rejection, 58% with a score ≥ 30 .

[0047] Using a nucleic acid technology or a protein technology to generate a gene expression profile, one of skill in the art would select the appropriate devices and methods based upon such factors as the particular immune disorder or transplant, ease and needed accuracy of measurement of each particular nucleic acid or protein, the number of primers, probe sets or antibodies in the diagnostic set, and the like. It is contemplated that a gene expression profile based on a small diagnostic set of steroid modulated nucleic acids can be produced on a low density array or a thermal gradient chip in a clinic or a doctor's office.

[0048] Knowing steroid modulated *in vivo* expression of nucleic acids or proteins and establishing a correlation between their expression and steroid responsiveness, one of skill in the art can use diagnostic sets of primers, probe sets, antibodies and the like to determine the steroid responsiveness of a particular individual. To establish such correlations, nucleic acid or protein expression will be measured multiple times, and statistical methods or algorithms will be applied to determine the reliability of the measurement and to establish a threshold for the correlation. Correlations can be determined using samples from steroid responsive subjects. For example, knowing the steroid modulated *in vivo* expression levels of nucleic acids and an established correlation between the expression levels of such nucleic acids and the steroid responsiveness of

a group of subjects being treated for transplant rejection, one of skill in the art can extrapolate the steroid responsiveness of a previously untested subject.

[0049] The responsiveness of a subject, based on nucleic acid expression, can be used design or to modify a treatment plan including types and amounts of immunosuppressants or steroids being administered; the dose, frequency and duration of treatment; weaning protocol, and the like. If a subject develops or shows resistance to a particular immunosuppressant, nucleic acid or protein expression and established correlations or profiles can be used to re-evaluate the subject's responsiveness and to revise the subject's treatment plan.

[0050] Reagents used to establish a gene expression profile include but are not limited to genes and their splice variants, amplicons, LNAs, oligonucleotides, peptide nucleic acids, primers, and probe sets that can be used in nucleic acid technologies; and proteins and their fragments, antibodies, and affinity reagents that can be used in protein technologies. These reagents can be used in assays or diagnostic kits to determine or monitor steroid responsiveness of a subject, to screen or monitor subjects for the development or flare of immune disorder or for transplant rejection, to design or evaluate a treatment protocol, and the like.

[0051] Assays or diagnostic kits based on the reagents and nucleic acid and protein technologies described herein can be used with a sample from a subject to diagnose, classify or rule out an immune disorder such as SLE or MS; to select a clinical trial, to predict flare, to detect immunosuppressant or steroid responsiveness, to determine efficacy of a potential therapeutic agent, to design treatment regimes, to monitor the status of the subject or the treatment regime. In one alternative, the diagnostic kit comprises an array of reagents; in another, probe sets for use in RT-PCR.

[0052] Pharmacogenomics is the study of an individual's response to a particular therapeutic agent, immunosuppressant or combinations thereof. In this context, response refers to whether a particular drug will work better for a subject with a particular immune disorder or transplant. The methods disclosed provide for assigning a subject to a clinical trial or treatment regime based on disease or transplant status (quiescent or flare for immune disorder, rejection or non-rejection for transplant). Pharmacogenomics is also important in determining the dosage of a therapeutic agent based on age, classification and status of the subject. Individual steroid responsiveness, dosage and even timing of administration must be taken into account relative to side effects or potential interactions of various therapeutic agents. Some potentially useful

therapeutic agents, immunosuppressants and steroids are listed in the definitions and/or claims.

[0053] All of the references cited are hereby incorporated by reference herein. This invention will be better understood by reference to the following non-limiting Examples which serve to demonstrate the use of nucleic acid and protein expression to evaluate steroid responsiveness in subjects, to optimize steroid dosage, to predict periods of non-rejection in subjects with transplants in order to reduce the number of invasive procedures, EMBs, TBBs, and the like.

Tables 1, 2 and 3 described in detail in the Examples are provided below.

Table 1

Cluster	Probe Id	Gene Symbol	Average		P-value		Gene Name
			P-value	Pearson	CARGO	LARGO	
1	A 24 P146211	HIST1H2BD	0.000002	0.85	1.71E-08	0.000257	histone 1, H2bd
1	A 23 P59069	HIST1H2BO	0.000006	0.86	1.77E-07	0.000175	histone 1, H2bo
1	A 23 P366216	HIST1H2BH	0.000008	0.83	8.15E-08	0.00073	histone 1, H2bh
1	A 24 P55148	HIST1H2BJ	0.000010	0.86	1.24E-06	8.55E-05	histone 1, H2bj
1	A 23 P30776	HIST1H2BE	0.000010	0.85	2.64E-07	0.000406	histone 1, H2be
1	A 23 P42178	HIST1H2BF	0.000012	0.85	7.09E-07	0.000195	histone 1, H2bf
1	A 23 P402081	HIST1H2BN	0.000013	0.84	5.66E-07	0.000315	histone 1, H2bn
1	A 24 P156911	HIST2H2BE	0.000015	0.86	8.42E-07	0.000269	histone 2, H2be
1	A 23 P8013	HIST1H2BL	0.000019	0.84	3.65E-06	9.88E-05	histone 1, H2bl
1	A 23 P111054	HIST1H2BB	0.000021	0.84	1.39E-06	0.000304	histone 1, H2bb
1	A 23 P93180	HIST1H2BC	0.000023	0.8	3.31E-07	0.00159	histone 1, H2bc
1	A 24 P152345	LOC391566	0.000026	0.76	2.73E-08	0.0247	Histone H2B.n
1	A 32 P57854		0.000026	0.84	1.35E-06	0.0005	DKFZp586A0722
1	A 24 P3783	HIST1H2BM	0.000030	0.79	2.39E-07	0.00379	histone 1, H2bm
1	A 23 P111041	HIST1H2BI	0.000031	0.8	5.68E-07	0.00166	histone 1, H2bi
1	A 23 P30020	PLA2G12A	0.000050	0.86	1.45E-05	0.000173	phospholipase A2, group XIIA
1	A 23 P218131	C14orf151	0.000057	0.87	5.99E-05	5.34E-05	chromosome 14 ORF 151
1	A 23 P332992	HIST3H2BB	0.000059	0.84	9.35E-06	0.000368	histone 3, H2bb
1	A 23 P256618	C6orf79	0.000074	0.8	3.98E-06	0.00136	chromosome 6 open reading frame 79
1	A 24 P10884	GRAP2	0.000074	0.76	2.09E-07	0.026	GRB2-related adaptor protein 2
1	A 23 P167997	HIST1H2BG	0.000079	0.78	2.72E-06	0.00229	histone 1, H2bg
1	A 32 P100439	Ells1	0.000086	0.88	3.41E-05	0.000215	hypothetical protein Ells1
1	A 24 P219785	CALM3	0.000086	0.79	8.90E-07	0.00837	calmodulin 3
1	A 24 P164718	MARCH2	0.000088	0.81	1.13E-05	0.000679	membrane-associated ring finger (C3HC4) 2

Cluster	Probe Id	Gene Symbol	Average		P-value		Gene Name
			P value	Pearson	CARGO	FLARGO	
1	A_23_P154065	TUBA1	0.000117	0.83	2.21E-05	0.000615	tubulin, alpha 1
1	A_23_P258093	AGPAT1	0.000147	0.83	5.21E-05	0.000415	1-acylglycerol-3-phosphate O-acyltransferase 1
1	A_23_P410312	FLJ40142	0.000151	0.77	2.23E-06	0.0102	FLJ40142 protein
1	A_23_P29124	GP1BB	0.000156	0.84	5.15E-05	0.000473	glycoprotein Ib, beta polypeptide
1	A_23_P62351	ARMCX6	0.000159	0.75	9.75E-07	0.0258	armadillo repeat containing, X-linked 6
1	A_23_P164047	MMD	0.000175	0.8	1.52E-05	0.00201	monocyte to macrophage differentiation-associated
1	A_23_P33683	MARCH2	0.000204	0.8	4.40E-05	0.00095	membrane-associated ring finger (C3HC4) 2
1	A_23_P154070	TUBA1	0.000210	0.78	3.37E-05	0.00131	tubulin, alpha 1
1	A_23_P502710	GAS2L1	0.000240	0.84	6.95E-05	0.000831	growth arrest-specific 2 like 1
1	A_32_P122754	MGC17337	0.000259	0.79	9.01E-06	0.00746	chromosome 9 ORF 30
1	A_23_P128598	TUBA2	0.000281	0.81	3.96E-05	0.00199	tubulin, alpha 2
1	A_23_P40470	H2BFS	0.000294	0.77	4.14E-06	0.0209	H2B histone family, member S
1	A_23_P2114	FLJ20625	0.000351	0.77	3.21E-05	0.00383	hypothetical protein FLJ20625
1	A_23_P366254	SLC10A3	0.000431	0.78	3.30E-05	0.00562	solute carrier family 10 member 3
1	A_23_P103981	HIST2H2AA	0.000447	0.72	9.79E-06	0.0204	histone 2, H2aa
1	A_23_P39684	TLK1	0.000485	0.74	7.46E-06	0.0315	tousled-like kinase 1
1	A_24_P929650		0.000499	0.68	3.37E-06	0.0739	
1	A_23_P151120	ACRBP	0.000512	0.79	4.40E-05	0.00595	acrosin binding protein
1	A_32_P61936		0.000515	0.78	2.89E-05	0.00919	clone IMAGE:5173389
1	A_24_P124957	RAB11A	0.000575	0.78	6.15E-05	0.00537	RAB11A
1	A_23_P156550	TREML1	0.000615	0.77	8.03E-05	0.00471	triggering receptor expressed on myeloid cells-like 1
1	A_32_P4814	FAM11A	0.000625	0.78	6.66E-05	0.00586	family with sequence similarity 11 member A
1	A_23_P116264	NRGN	0.000664	0.76	3.83E-05	0.0115	neurogranin
1	A_24_P10657	CTL2	0.000725	0.75	0.000104	0.00505	solute carrier family 44 member 2

Cluster	Probe Id	Gene Symbol	Average		Pvalue		Gene Name
			P value	Pearson	CARGO	LARGO	
1	A_23_P330611	WASPIP	0.000828	0.75	0.00017	0.00403	Wiskott-Aldrich syndrome protein interacting protein
1	A_23_P165840	ODC1	0.000840	0.72	6.98E-05	0.0101	ornithine decarboxylase 1
1	A_23_P40718	PARVB	0.000912	0.68	1.68E-05	0.0495	parvin, beta
1	A_23_P166677	MFSD1	0.000996	0.76	0.000126	0.00788	major facilitator superfamily domain containing 1
1	A_23_P54488	BG1	0.001028	0.75	5.34E-05	0.0198	acyl-CoA synthetase bubblegum family member 1
1	A_23_P78209	MAFG	0.001108	0.74	0.000307	0.004	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G
1	A_24_P74371	PPGB	0.001355	0.74	0.000157	0.0117	protective protein for beta-galactosidase
1	A_24_P259490	ARF1	0.001465	0.6	9.37E-06	0.229	ADP-ribosylation factor 1
1	A_23_P118038	NUTF2	0.001469	0.73	0.000785	0.00275	nuclear transport factor 2
1	A_23_P98900	FLJ22471	0.001471	0.73	0.000125	0.0173	limkain beta 2
1	A_23_P31177	FLJ11000	0.001491	0.67	6.11E-05	0.0364	hypothetical protein FLJ11000
1	A_24_P74374	PPGB	0.001500	0.73	2.25E-05	0.1	protective protein for beta-galactosidase
1	A_24_P825942		0.001557	0.64	1.05E-05	0.231	FLJ10934 fis
1	A_24_P107695	ACTN1	0.001690	0.76	0.00121	0.00236	actinin, alpha 1
1	A_23_P147098	MTPN	0.001788	0.7	0.000283	0.0113	myotrophin
1	A_32_P194848	TAGLN2	0.002650	0.65	5.53E-05	0.127	transgelin 2
1	A_32_P75141		0.003041	0.74	0.00108	0.00856	
1	A_23_P76364	CD9	0.003060	0.73	0.000466	0.0201	CD9 antigen
1	A_23_P255444	DAPP1	0.003114	0.61	3.20E-05	0.303	dual adaptor of phosphotyrosine and 3-phosphoinositides
1	A_23_P102109	TUBA4	0.003144	0.73	0.000308	0.0321	tubulin, alpha 4
1	A_24_P55465	MTPN	0.003519	0.64	7.94E-05	0.156	myotrophin
1	A_23_P502224	DIA1	0.003590	0.68	0.000651	0.0198	cytochrome b5 reductase 3

Cluster	Probe Id	Gene Symbol	Average		P value		Gene Name
			P-value	Pearson	CARGO	LARGO	
1	A_23_P100469	TXNL4B	0.003717	0.67	0.000225	0.0614	thioredoxin-like 4B
1	A_23_P138717	RGS10	0.003966	0.69	0.0011	0.0143	regulator of G-protein signalling 10
1	A_23_P162559	SPPL3	0.004619	0.7	0.00983	0.00217	signal peptide peptidase 3
1	A_24_P137897	IFRD1	0.004658	0.66	0.000716	0.0303	interferon-related developmental regulator 1
1	A_24_P147263	USP31	0.005096	0.69	0.000757	0.0343	ubiquitin specific peptidase 31
1	A_23_P361773	CCND3	0.005943	0.65	0.000678	0.0521	cyclin D3
1	A_23_P305711	RYBP	0.006042	0.68	0.000723	0.0505	RING1 and YY1 binding protein
1	A_32_P192545	LOC158931	0.006442	0.66	0.000497	0.0835	transcription elongation factor A (SII)-like 6
1	A_23_P141394	WIP149	0.007588	0.61	0.0236	0.00244	WD repeat domain, phosphoinositide interacting 1
1	A_23_P341392	MGC32124	0.007797	0.67	0.0032	0.019	hypothetical protein MGC32124
1	A_23_P138881	ACTN3	0.007808	0.64	0.00127	0.048	actinin, alpha 3
1	A_23_P434442	TCEAL3	0.008260	0.67	0.00071	0.0961	transcription elongation factor A (SII)-like 3
1	A_23_P302550	RGS18	0.009014	0.74	0.00549	0.0148	regulator of G-protein signalling 18
1	A_23_P30799	HIST1H3F	0.009138	0.62	0.0113	0.00739	histone 1, H3f
1	A_24_P80135	PTPN18	0.009658	0.62	0.000691	0.135	protein tyrosine phosphatase, non-receptor type 18
1	A_24_P319736	MEIS1	0.010057	0.67	0.0165	0.00613	myeloid ecotropic viral integration site 1 homolog
1	A_23_P6321	CLDN5	0.011739	0.62	0.0432	0.00319	claudin 5
1	A_24_P186414	TEX27	0.013616	0.5	0.000574	0.323	zinc finger, AN1-type domain 3
1	A_23_P215479	CYLN2	0.015169	0.58	0.00133	0.173	cytoplasmic linker 2
1	A_23_P360379	EGLN3	0.015179	0.57	0.00144	0.16	egl nine homolog 3
1	A_23_P95470	CD151	0.016468	0.54	0.000565	0.48	CD151 antigen
1	A_24_P29733	PFTK1	0.021128	0.59	0.0036	0.124	PFTAIRE protein kinase 1

Cluster	Probe/Id	Gene Symbol	Average		P value		Gene Name
			P value	Pearson	CARGO	LEARGO	
1	A_24_P333525	RABGAP1L	0.021667	0.56	0.00144	0.326	RAB GTPase activating protein 1-like
1	A_23_P200325	RABGAP1L	0.024429	0.55	0.0016	0.373	RAB GTPase activating protein 1-like
1	A_23_P502915	WDR1	0.024880	0.52	0.00259	0.239	WD repeat domain 1
1	A_23_P132226	TPST2	0.025788	0.56	0.0025	0.266	tyrosylprotein sulfotransferase 2
1	A_24_P922357	LOC128977	0.027026	0.53	0.00658	0.111	hypothetical protein LOC128977
1	A_23_P141688	RAB31	0.029041	0.58	0.0451	0.0187	RAB31
1	A_24_P134834	DKFZp547E052	0.030231	0.49	0.0037	0.247	hypothetical protein LOC84236
1	A_24_P179339		0.030626	0.55	0.0111	0.0845	humanin
1	A_23_P126135	MFN2	0.030828	0.54	0.0337	0.0282	mitofusin 2
1	A_24_P244916	SERF2	0.032581	0.47	0.00386	0.275	small EDRK-rich factor 2
1	A_23_P141974	TPM4	0.032790	0.54	0.0048	0.224	tropomyosin 4
1	A_23_P251825	IFRD1	0.032850	0.6	0.0188	0.0574	interferon-related developmental regulator 1
1	A_23_P48175	MGC5576	0.035875	0.57	0.055	0.0234	transmembrane protein 106C
1	A_32_P119165		0.037532	0.57	0.0156	0.0903	
1	A_32_P42780		0.041711	0.51	0.0578	0.0301	
1	A_23_P502913	WDR1	0.041857	0.54	0.02	0.0876	WD repeat domain 1
1	A_23_P27207	SCGB1C1	0.045425	0.49	0.114	0.0181	secretoglobulin, family 1C member 1
1	A_24_P403303	PHF20L1	0.046385	0.5	0.0132	0.163	PHD finger protein 20-like 1
1	A_24_P316059		0.051220	0.55	0.045	0.0583	
1	A_23_P147199	ZNF271	0.066915	0.4	0.0116	0.386	zinc finger protein 271
1	A_32_P55979		0.080677	0.45	0.0576	0.113	6-pyruvoyltetrahydropterin synthase
1	A_32_P163089	LOC387882	0.095436	0.45	0.012	0.759	hypothetical protein
1	A_23_P2661	RAP1B	0.382999	0.26	0.384	0.382	RAP1B
1	A_23_P154294	MGC13005	0.440704	-0.12	0.195	0.996	FLJ44010 fis
2	A_23_P210330		0.000031	0.88	2.72E-05	3.45E-05	CS0DL009YB17 of B cells

Cluster	ProbeId	GeneSymbol	Average		P value		GeneName
			P value	Pearson	CARGO	LARGO	
2	A_24_P365901	MGC50844	0.000038	0.87	2.91E-06	0.000507	tetraspanin 33
2	A_24_P226322	SH3BGRL2	0.000048	0.85	6.74E-06	0.000337	SH3 domain binding glutamic acid-rich protein like 2
2	A_23_P152906	ALOX12	0.000071	0.86	8.47E-06	0.000587	arachidonate 12-lipoxygenase
2	A_24_P148321	HIST2H2BE	0.000071	0.77	6.88E-07	0.00738	histone 2, H2be
2	A_23_P256205	ABLIM3	0.000074	0.83	8.81E-06	0.000629	actin binding LIM protein family member 3
2	A_24_P209171	SH3BGRL2	0.000081	0.84	1.39E-05	0.000471	SH3 domain binding glutamic acid-rich protein like 2
2	A_23_P390006	PCSK6	0.000134	0.77	5.81E-06	0.00309	proprotein convertase subtilisin/kexin type 6
2	A_23_P129221	FAH	0.000137	0.7	1.83E-07	0.102	fumarylacetoacetate hydrolase
2	A_23_P430818	HSPC159	0.000152	0.82	0.000114	0.000202	HSPC159 protein
2	A_24_P218905	NET-5	0.000162	0.8	4.72E-05	0.000556	tetraspanin 9
2	A_32_P145477		0.000218	0.84	5.04E-05	0.000947	BX350256
2	A_24_P290188		0.000233	0.81	0.000152	0.000357	
2	A_24_P706752		0.000244	0.83	4.95E-05	0.0012	
2	A_23_P143720	GRAP2	0.000276	0.77	4.55E-06	0.0168	GRB2-related adaptor protein 2
2	A_23_P77971	ITGA2B	0.000315	0.78	2.23E-05	0.00445	integrin, alpha 2b
2	A_23_P212436	CTDSPL	0.000394	0.82	3.79E-05	0.0041	carboxy-terminal domain, RNA polymerase II, polypeptide A
2	A_23_P152926	GP1BA	0.000398	0.85	0.00144	0.00011	glycoprotein Ib, alpha polypeptide
2	A_24_P189997	PCSK6	0.000403	0.69	4.78E-06	0.034	proprotein convertase subtilisin/kexin type 6
2	A_23_P38519	ITGB3	0.000408	0.81	0.000111	0.0015	integrin, beta 3
2	A_24_P64167	PTGSI	0.000411	0.78	3.30E-05	0.00513	prostaglandin-endoperoxide synthase 1
2	A_24_P318656	ITGB3	0.000422	0.83	0.000323	0.000552	integrin, beta 3
2	A_23_P2414		0.000447	0.73	1.24E-05	0.0161	PSEC0021 fis

Cluster	Probe Id	Gene Symbol	Average		P value		Gene Name	
			P value	Pearson	CARGO	ELARGO		
2	A_23_P216966	PTGS1	0.000455	0.78	2.95E-05	0.00703	prostaglandin-endoperoxide synthase 1	
2	A_32_P17743		0.000470	0.79	0.000174	0.00127		
2	A_23_P79978	SLC24A3	0.000581	0.78	4.70E-05	0.00718	solute carrier family 24 member 3	
2	A_23_P43810	LTBP1	0.000582	0.8	0.000185	0.00183	latent transforming growth factor beta binding protein 1	
2	A_23_P6034	TUBB1	0.000583	0.8	4.83E-05	0.00704	tubulin, beta 1	
2	A_24_P176079	WASF3	0.000620	0.76	0.00542	7.10E-05	WAS protein family member 3	
2	A_23_P202823	CTTN	0.000655	0.76	0.00245	0.000175	cortactin	
2	A_23_P210358	LIMS1	0.000690	0.79	0.000229	0.00208	LIM and senescent cell antigen-like domains 1	
2	A_24_P929003	ITGB3	0.000715	0.8	0.000133	0.00384	integrin, beta 3	
2	A_23_P389118	TMEM16F	0.000766	0.72	1.73E-05	0.0339	DKFZp313M0720	
2	A_23_P106042	CKLFSF5	0.000769	0.78	0.000211	0.0028	CKLF-like MARVEL transmembrane domain containing 5	
2	A_24_P160104	TUBA8	0.000797	0.76	0.000232	0.00274	tubulin, alpha 8	
2	A_23_P207507	ABCC3	0.000809	0.77	0.000122	0.00536	ATP-binding cassette, sub-family C member 3	
2	A_23_P102731	SMOX	0.000819	0.75	8.77E-05	0.00764	spermine oxidase	
2	A_32_P137604		0.000838	0.81	0.000348	0.00202	clone IMAGE:3869276	
2	A_23_P104624	KIAA0830	0.000910	0.75	0.000219	0.00378	KIAA0830 protein, partial cds	
2	A_23_P359277		0.000965	0.76	6.65E-05	0.014	ELOVL family member 7	
2	A_23_P151133	NET-5	0.001003	0.75	0.000115	0.00875	tetraspanin 9	
2	A_23_P105957	ACTN1	0.001008	0.78	0.000899	0.00113	actinin, alpha 1	
2	A_23_P17095	TFPI	0.001031	0.72	0.00136	0.000782	tissue factor pathway inhibitor	
2	A_23_P25974	TTC7B	0.001071	0.81	0.000634	0.00181	tetratricopeptide repeat domain 7B	
2	A_32_P168342	C6orf25	0.001113	0.78	0.00016	0.00774	FLJ35073 fis	
2	A_23_P215913	CLU	0.001147	0.8	0.000129	0.0102	clusterin	

Cluster	Probe Id	Gene Symbol	Average		P-value		Gene Name
			P-value	Pearson	CARGO	LARGO	
2	A_23_P416581	GNAZ	0.001155	0.8	0.000335	0.00398	guanine nucleotide binding protein
2	A_24_P122337	SYTL4	0.001175	0.74	0.000107	0.0129	synaptotagmin-like 4
2	A_23_P166633	ITGB5	0.001207	0.79	0.000729	0.002	integrin, beta 5
2	A_24_P185186	LOC201191	0.001257	0.71	2.60E-05	0.0608	sterile alpha motif domain containing 14
2	A_24_P333372		0.001295	0.72	0.000492	0.00341	FLJ35984 fis
2	A_23_P217998	JAM3	0.001310	0.73	5.66E-05	0.0303	junctional adhesion molecule 3
2	A_23_P81930	C6orf25	0.001357	0.61	7.97E-06	0.231	chromosome 6 ORF 25
2	A_23_P152160	SNN	0.001428	0.75	0.000294	0.00694	stannin
2	A_23_P109974	RAB6B	0.001475	0.71	0.0035	0.000622	RAB6B
2	A_23_P45524	NGFRAP1	0.001761	0.75	0.000886	0.0035	nerve growth factor receptor associated protein 1
2	A_23_P7642	SPARC	0.001769	0.74	0.00031	0.0101	secreted protein, acidic, cysteine-rich
2	A_23_P73457	RUFY1	0.001850	0.77	0.0021	0.00163	RUN and FYVE domain containing 1
2	A_32_P136450		0.001905	0.58	2.20E-05	0.165	AF220206 Nedd4 WW domain-binding protein 2
2	A_23_P17724	SEP5	0.002039	0.6	2.52E-05	0.165	septin 5
2	A_23_P42975	PRKAR2B	0.002098	0.76	0.000284	0.0155	protein kinase, cAMP-dependent, regulatory, type II, beta
2	A_23_P19987	IMP-3	0.002357	0.75	0.000157	0.0354	IGF-II mRNA-binding protein 3
2	A_32_P162250	ARHGAP18	0.002510	0.75	0.00348	0.00181	Rho GTPase activating protein 18
2	A_24_P251534	CTDSPL	0.002766	0.78	0.00126	0.00607	carboxy-terminal domain, RNA polymerase II, polypeptide A
2	A_23_P391586		0.002833	0.73	0.000818	0.00981	tropomyosin 1 transcript variant 3
2	A_24_P319923	MYLK	0.003091	0.72	0.00281	0.0034	myosin, light polypeptide kinase
2	A_24_P13190	ESAM	0.003222	0.72	0.00145	0.00716	endothelial cell adhesion molecule

Cluster	Probe Id	Gene Symbol	Average		P value		P value		Gene Name
			P value	Pearson	CARCO	LARGO	CARCO	LARGO	
2	A_23_P105562	VWF	0.003247	0.68	0.000172	0.0613	0.000172	0.0613	von Willebrand factor
2	A_23_P111701	GNG11	0.003249	0.67	0.00536	0.00197	0.00536	0.00197	guanine nucleotide binding protein, gamma 11
2	A_24_P254850	KIAA0420	0.003707	0.74	0.000387	0.0355	0.000387	0.0355	KIAA0420 mRNA
2	A_24_P79403	PF4	0.003837	0.69	0.00162	0.00909	0.00162	0.00909	platelet factor 4
2	A_23_P121596	PPBP	0.004165	0.68	0.00064	0.0271	0.00064	0.0271	pro-platelet basic protein
2	A_23_P143817	MYLK	0.004643	0.7	0.0055	0.00392	0.0055	0.00392	myosin, light polypeptide kinase
2	A_23_P217428	ARHGAP6	0.004934	0.72	0.00412	0.00591	0.00412	0.00591	Rho GTPase activating protein 6
2	A_23_P146584	MGC17337	0.005001	0.74	0.00421	0.00594	0.00421	0.00594	chromosome 9 ORF 30
2	A_23_P149992	PDLIM1	0.005416	0.57	7.66E-05	0.383	7.66E-05	0.383	PDZ and LIM domain 1
2	A_23_P500844	PDE5A	0.005427	0.67	0.000425	0.0693	0.000425	0.0693	phosphodiesterase 5A, cGMP-specific
2	A_23_P99906	HOMER2	0.006003	0.69	0.000615	0.0586	0.000615	0.0586	homer homolog 2
2	A_24_P921366	CALD1	0.006314	0.68	0.000291	0.137	0.000291	0.137	caldesmon 1
2	A_23_P125233	CNN1	0.006368	0.63	0.000524	0.0774	0.000524	0.0774	calponin 1, basic
2	A_23_P8906	LRP12	0.006827	0.7	0.00295	0.0158	0.00295	0.0158	low density lipoprotein-related protein 12
2	A_32_P140139	F13A1	0.007655	0.67	0.002	0.0293	0.002	0.0293	coagulation factor XIII, A1 polypeptide
2	A_23_P360804	CPNE5	0.008140	0.6	0.00162	0.0409	0.00162	0.0409	copine V
2	A_24_P188071	TUBA6	0.012196	0.59	0.037	0.00402	0.037	0.00402	tubulin, alpha 6
2	A_23_P137697	SELP	0.013649	0.63	0.00786	0.0237	0.00786	0.0237	selectin P
2	A_24_P892612		0.016210	0.64	0.00457	0.0575	0.00457	0.0575	DKFZp313A137
2	A_23_P48212	CLEC1B	0.020106	0.66	0.00607	0.0666	0.00607	0.0666	C-type lectin domain family 1, member B
2	A_23_P58396	PDGFC	0.039597	0.59	0.0564	0.0278	0.0564	0.0278	platelet derived growth factor C
2	A_23_P209527		0.040457	0.56	0.0186	0.088	0.0186	0.088	A31642 villin
2	A_23_P168556	STX1A	0.050571	0.53	0.0444	0.0576	0.0444	0.0576	syntaxin 1A
2	A_32_P18723	DKFZp762C1112	0.051355	0.52	0.0298	0.0885	0.0298	0.0885	FLJ38153 fis
2	A_23_P52207	BAMBI	0.057018	0.46	0.00791	0.411	0.00791	0.411	BMP and activin membrane-

Cluster	Probe Id	Gene Symbol	P Value	Average Pearson	P Value	CORGO	P value	Gene Name
2	A_23_P431388	SPOCD1	0.068662	0.52	0.0941	0.0501	0.0501	bound inhibitor homolog
2	A_23_P371266	DNM3	0.072500	0.49	0.0688	0.0764	0.0764	SPOC domain containing 1
2	A_32_P179138		0.087964	0.43	0.186	0.0416	0.0416	dynamins 3
3	A_23_P111267	SH3BGR2	0.000145	0.84	5.79E-05	0.000364	0.000364	clone IMAGE:5302158
3	A_23_P219045	HIST1H3D	0.000158	0.76	4.14E-06	0.00601	0.00601	SH3 domain binding glutamic acid-rich protein like 2
3	A_24_P315256		0.000195	0.64	2.00E-07	0.19	0.19	histone 1, H3d
3	A_23_P91423	C20orf112	0.000206	0.77	2.21E-05	0.00192	0.00192	chromosome 20 ORF 112
3	A_23_P149545	HIST2H2BE	0.000234	0.75	7.28E-06	0.00752	0.00752	histone 2, H2be
3	A_23_P84448	TUBA4	0.000329	0.65	8.46E-07	0.128	0.128	tubulin, alpha 4
3	A_23_P405295	LCE3C	0.000333	0.8	8.47E-05	0.00131	0.00131	late cornified envelope 3C
3	A_23_P152909	ALOX12	0.000375	0.79	0.000108	0.0013	0.0013	arachidonate 12-lipoxygenase
3	A_23_P210939	ITGB4BP	0.000474	0.76	0.00022	0.00102	0.00102	integrin beta 4 binding protein
3	A_23_P4944	CALM3	0.000497	0.64	2.13E-06	0.116	0.116	calmodulin 3
3	A_32_P221799	HIST1H2AM	0.000511	0.79	0.000133	0.00196	0.00196	histone 1, H2am
3	A_23_P436138	MAX	0.000609	0.66	5.84E-06	0.0636	0.0636	MYC associated factor X
3	A_24_P180680	LAPTM4B	0.000732	0.72	2.30E-05	0.0233	0.0233	lysosomal associated protein transmembrane 4 beta
3	A_24_P753476	LOC340508	0.000758	0.8	0.000111	0.00518	0.00518	LOC340508
3	A_24_P65373	ITGA2B	0.000988	0.64	5.64E-06	0.173	0.173	integrin, alpha 2b
3	A_24_P918032	LOC339005	0.001007	0.74	9.75E-05	0.0104	0.0104	LOC339005
3	A_23_P160546	FLJ11280	0.001239	0.76	0.000919	0.00167	0.00167	family with sequence similarity 63, member A
3	A_23_P41280	PAICS	0.001436	0.72	0.00562	0.000367	0.000367	phosphoribosylaminoimidazole carboxylase
3	A_24_P258633	TUBB3	0.001463	0.08	0.000723	0.00296	0.00296	tubulin, beta 3
3	A_24_P308506	CML2	0.001503	0.73	0.00753	0.0003	0.0003	putative N-acetyltransferase Camello 2
3	A_23_P206212	THBS1	0.001565	0.72	0.000123	0.0199	0.0199	thrombospondin 1

Cluster	Probe Id	Gene Symbol	Average		P value		Gene Name
			P value	Pearson	CARGO	LARGO	
3	A_24_P382637	GTPBP5	0.001741	0.7	0.00549	0.000552	GTP binding protein 5
3	A_32_P3385		0.001798	0.72	0.00358	0.000903	CS0DI060YD22
3	A_23_P156708	TNXB	0.001969	0.68	0.043	9.02E-05	tenascin XB
3	A_23_P74138	TAGLN2	0.002092	0.66	5.86E-05	0.0747	transgelin 2
3	A_23_P215735	ST7	0.002094	0.6	1.72E-05	0.255	suppression of tumorigenicity 7
3	A_23_P113701	PDGFA	0.002236	0.74	0.000365	0.0137	platelet-derived growth factor
3	A_23_P121564	GUCY1B3	0.002640	0.62	2.63E-05	0.265	guanylate cyclase 1, soluble, beta 3
3	A_24_P189533	KIAA0830	0.002796	0.72	0.000774	0.0101	KIAA0830
3	A_32_P89709		0.002878	0.72	0.000991	0.00836	tropomyosin 1
3	A_23_P15647	NLK	0.003035	0.64	5.98E-05	0.154	nemo-like kinase
3	A_23_P24616	CSE-C	0.003070	0.67	0.00016	0.0589	sialic acid acetyltransferase
3	A_23_P73239	NCKAP1	0.003580	0.67	0.000543	0.0236	NCK-associated protein 1
3	A_23_P3946	NT5M	0.003733	0.48	2.66E-05	0.524	5',3'-nucleotidase, mitochondrial
3	A_23_P19624	BMP6	0.004005	0.69	0.00225	0.00713	bone morphogenetic protein 6
3	A_24_P926709		0.004013	0.61	0.00189	0.00852	
3	A_23_P90407	CASP14	0.004129	0.7	0.0084	0.00203	caspase 14
3	A_23_P167096	VEGFC	0.004234	0.67	0.000199	0.0901	vascular endothelial growth factor C
3	A_23_P421843	LOC201191	0.004449	0.57	4.90E-05	0.404	sterile alpha motif domain containing 14
3	A_23_P501831	C5orf4	0.004848	0.65	0.000569	0.0413	chromosome 5 ORF 4
3	A_23_P417942	FNBP1L	0.005106	0.63	0.000349	0.0747	formin binding protein 1-like
3	A_23_P307525	ANKRD9	0.005136	0.52	6.28E-05	0.42	ankyrin repeat domain 9
3	A_32_P92212		0.005401	0.62	0.000204	0.143	IMAGE:3271727
3	A_23_P156284	DBN1	0.005544	0.67	0.000456	0.0674	drebrin 1
3	A_23_P18539	MMRN1	0.005781	0.57	0.000205	0.163	multimerin 1
3	A_24_P38387	NDRG1	0.005842	-0.6	0.00555	0.00615	N-myc downstream regulated gene 1
3	A_23_P155979	EGF	0.006397	0.67	0.00132	0.031	epidermal growth factor (beta-

Cluster	Probe/Id	Gene Symbol	Average		P value		Gene Name
			P value	Pearson	CARGO	LARGO	
3	A_23_P401361	PITPNM2	0.007348	0.62	0.00036	0.15	phosphatidylinositol transfer protein, membrane-associated 2
3	A_24_P385313	PTPRF	0.008340	0.64	0.00778	0.00894	protein tyrosine phosphatase, receptor type, F
3	A_23_P141055	TGFB1I1	0.008607	0.48	9.89E-05	0.749	transforming growth factor beta 1 induced transcript 1
3	A_24_P204257		0.011918	0.65	0.00419	0.0339	
3	A_23_P369899	RIS1	0.013687	0.45	0.000409	0.458	Ras-induced senescence 1
3	A_24_P167654	SLC8A3	0.016278	0.57	0.00207	0.128	solute carrier family 8 member 3
3	A_24_P405981		0.018386	0.58	0.00313	0.108	CS0DD001YH15
3	A_23_P431853		0.018431	0.6	0.0114	0.0298	A-COL04217
3	A_23_P367043	MGC26484	0.020359	0.61	0.0132	0.0314	CDC14 cell division cycle 14 homolog C
3	A_23_P135499	CLIC4	0.026410	0.54	0.0094	0.0742	chloride intracellular channel 4
3	A_24_P32473		0.026587	0.51	0.00198	0.357	ELOVL family member 7
3	A_23_P81934	C6orf25	0.031498	0.53	0.00439	0.226	chromosome 6 ORF 25
3	A_24_P414999	LAPTM4B	0.032256	0.55	0.00439	0.237	lysosomal associated protein transmembrane 4 beta
3	A_23_P207414	MGC2744	0.034782	0.36	0.00143	0.846	alanyl-tRNA synthetase domain containing 1
3	A_32_P141437		0.039100	0.51	0.0104	0.147	FKSG73
3	A_32_P59262		0.057092	0.52	0.0205	0.159	IMAGE:3104077
3	A_23_P61945	MITF	0.061935	0.51	0.028	0.137	microphthalmia-associated transcription factor
3	A_23_P16866	VIL1	0.066077	0.48	0.114	0.0383	villin 1
3	A_23_P127642	ARHGEF12	0.072900	0.48	0.0146	0.364	Rho guanine nucleotide exchange factor 12
3	A_24_P713185		0.075192	0.46	0.0287	0.197	IMAGE:4271522
3	A_23_P69573	GUCY1A3	0.106153	0.38	0.572	0.0197	guanylate cyclase 1, soluble,

Cluster	ProbeId	Gene Symbol	Average		P Value		Gene Name
			P value	Pearson	CARGO	LARGO	
3	A_23_P257871	DAB2	0.137768	0.43	0.146	0.13	alpha 3
3	A_24_P331882	KIAA1211	0.188615	0.35	0.0604	0.589	disabled homolog 2, mitogen-responsive phosphoprotein
3	A_23_P154526	GRB14	0.205232	0.32	0.432	0.0975	DKFZp434F117
3	A_23_P45304	XK	0.275935	0.2	0.162	0.47	growth factor receptor-bound protein 14
3	A_23_P104493	PAPSS2	0.588394	0.12	0.349	0.992	Kell blood group precursor
4	A_24_P143440	DNCL2A	0.000004	0.85	2.56E-08	0.000546	3'-phosphoadenosine 5'-phosphosulfate synthase 2
4	A_23_P208788	C19orf33	0.000040	0.85	0.000182	8.84E-06	dynein, light chain, roadblock-type 1
4	A_24_P68631	HIST2H2AB	0.000048	0.83	1.11E-05	0.000204	chromosome 19 ORF 33
4	A_23_P120364	C20orf149	0.000060	0.8	9.45E-07	0.00385	histone 2, H2ab
4	A_23_P202029	SPFH1	0.000105	0.81	0.000107	0.000104	chromosome 20 ORF 149
4	A_24_P287075	MAP4K2	0.000111	0.8	4.13E-06	0.00296	SPFH domain family, member 1
4	A_23_P149301	HIST3H2A	0.000111	0.83	1.06E-05	0.00117	mitogen-activated protein kinase
4	A_24_P6921		0.000288	0.8	0.000193	0.00043	kinase kinase kinase 2
4	A_24_P135801		0.000314	0.8	7.96E-05	0.00124	histone 3, H2a
4	A_24_P45767	FLJ21839	0.000417	0.8	0.00074	0.000235	LOC541471 protein
4	A_23_P42375	RAB32	0.000526	0.76	9.01E-05	0.00307	CS0DF024Y114
4	A_23_P354705	ST8SIA1	0.000559	-0.66	0.000185	0.00169	FLJ21839
4	A_23_P407565	CX3CR1	0.000593	-0.64	0.00883	3.98E-05	RAB32
4	A_23_P59045	HIST1H2AE	0.000607	0.77	0.000123	0.003	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1
4	A_24_P911960		0.000642	0.77	7.30E-05	0.00565	chemokine receptor 1
4	A_32_P184937		0.000655	0.77	0.000585	0.000733	histone 1, H2ae
4	A_23_P138117	CAMTA1	0.000678	0.76	0.000183	0.00251	IMAGE:1699732
							BU678941
							calmodulin binding transcription activator 1

Cluster	ProbeId	Gene Symbol	Average P-value	Pearson	P-value	Gene Name
4	A_32_P3113	2-Mar	0.000802	0.79	0.000119	membrane-associated ring finger (C3HC4) 2
4	A_32_P54137	UQCRH	0.001063	0.74	0.00576	ubiquinol-cytochrome c reductase hinge protein
4	A_23_P396626	APIGBP1	0.001214	-0.66	0.00201	API gamma subunit binding protein 1
4	A_24_P227927	IL21R	0.001223	-0.62	0.00571	interleukin 21 receptor
4	A_23_P110167	MGST2	0.001371	0.78	0.00108	microsomal glutathione S-transferase 2
4	A_23_P120933	ATF4	0.001416	0.72	0.00105	activating transcription factor 4
4	A_23_P55706	RELB	0.001465	-0.64	0.00511	v-rel reticuloendotheliosis viral oncogene homolog B
4	A_24_P323835	H3F3A	0.001573	0.71	0.00029	H3 histone, family 3A
4	A_24_P273143	MGC4677	0.001656	0.74	0.00294	hypothetical protein MGC4677
4	A_23_P111037	HIST1H3A	0.001703	0.74	0.00107	histone 1, H3a
4	A_24_P223384	HIST1H2AB	0.001843	0.72	0.000724	histone 1, H2ab
4	A_23_P132285		0.001983	0.74	0.00257	mercaptopurinate sulfortransferase
4	A_23_P52101	NQO3A2	0.002328	0.72	0.00417	cytochrome b5 reductase 1
4	A_24_P608790		0.002619	0.7	0.00381	
4	A_24_P122732	SLC41A1	0.002673	-0.63	0.000901	solute carrier family 41, member 1
4	A_32_P132317		0.003079	-0.63	0.00139	
4	A_23_P218817	CPT1B	0.003379	-0.63	0.0066	carnitine palmitoyltransferase 1B
4	A_32_P132169		0.003419	0.7	0.00191	
4	A_24_P102769	UQCRH	0.003712	0.69	0.00501	ubiquinol-cytochrome c reductase hinge protein
4	A_23_P148410	FTHL17	0.005296	0.51	6.36E-05	ferritin, heavy polypeptide-like 17
4	A_23_P214330	SERPINB1	0.005642	0.64	0.00584	serpin peptidase inhibitor, clade

Cluster	Probe Id	Gene Symbol	P value	Average Pearson	P value	CARGO	P value	Gene Name
								B, member 1
4	A 32_P94521		0.005688	0.68	0.000795	0.0407		
4	A 32_P59302		0.006040	0.61	0.00519	0.00703		IMAGE:6254031
4	A 23_P121082	GBE1	0.006706	0.67	0.0048	0.00937		glucan branching enzyme 1
4	A 24_P7934		0.007472	0.62	0.00871	0.00641		LOC391769
4	A 23_P250671	GPX1	0.044571	0.46	0.0413	0.0481		glutathione peroxidase 1
4	A 23_P69218	LOC55831	0.047663	0.48	0.0292	0.0778		transmembrane protein 111
4	A 24_P913629		0.083624	0.43	0.0222	0.315		
5	A 23_P122007	LOC90355	0.000013	0.78	5.58E-07	0.000291		hypothetical gene supported by AF038182
5	A 23_P210060	MGC13057	0.000032	0.88	2.36E-06	0.000436		DKFZp686I15210
5	A 23_P138417	RSU1	0.000035	0.8	1.88E-07	0.0066		Ras suppressor protein 1
5	A 23_P350591	CXorf20	0.000038	0.83	7.91E-07	0.00181		chromosome X ORF 20
5	A 23_P114275	PGRMC1	0.000052	0.75	2.08E-07	0.0131		progesterone receptor membrane component 1
5	A 24_P362540	DDEF2	0.000137	0.84	1.92E-05	0.000971		development and differentiation enhancing factor 2
5	A 23_P167983	HIST1H2AC	0.000193	0.78	5.63E-06	0.0066		histone 1, H2ac
5	A 23_P103070	YWHAH	0.000223	0.8	9.09E-06	0.00546		tyrosine 3-monooxygenase
5	A 24_P273666	GNAS	0.000238	0.74	4.23E-06	0.0134		GNAS complex locus
5	A 23_P333484	HIST1H3H	0.000275	0.79	0.000139	0.000545		histone 1, H3h
5	A 23_P407203		0.000286	0.66	4.61E-07	0.177		FLJ42816 fis
5	A 23_P414273	NID67	0.000314	0.82	0.000841	0.000117		MSTP150
5	A 23_P102391	SLC40A1	0.000325	0.76	1.69E-05	0.00624		solute carrier family 40 member 1
5	A 23_P206018		0.000403	0.76	1.69E-05	0.00963		tropomyosin 1
5	A 23_P72668	SDPR	0.000567	0.78	8.47E-05	0.00379		serum deprivation response
5	A 24_P228550	TUBB1	0.000606	0.82	0.000181	0.00203		tubulin, beta 1
5	A 23_P107612	RAB27B	0.000744	0.8	0.000151	0.00367		RAB27B
5	A 23_P77145	RAB11A	0.000893	0.76	0.000114	0.007		RAB11A
5	A 23_P502797	WDFY1	0.000895	-0.01	0.00393	0.000204		WD repeat and FYVE domain

Cluster	ProbeId	GeneSymbol	Average P value	Pearson P value	CARGO P value	GeneName
5	A_23_P211910	PLOD2	0.000943	0.77	0.000532	containing 1
5	A_24_P44462	TPM1	0.000991	0.73	4.86E-05	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2
5	A_32_P125917		0.001068	0.79	0.000144	tropomyosin 1
5	A_23_P157128	SCAP2	0.001259	0.72	0.00013	BF238843
5	A_32_P168349	C6orf25	0.001322	0.74	9.06E-05	src family associated phosphoprotein 2
5	A_23_P216679	CDC14B	0.001372	0.66	1.61E-05	FLJ35073 fis
5	A_23_P63371	TAL1	0.001478	0.8	0.00208	CDC14 cell division cycle 14 homolog B
5	A_23_P12884	GRK5	0.001545	0.73	6.47E-05	T-cell acute lymphocytic leukemia 1
5	A_23_P126836	TNFSF4	0.001611	0.78	0.000238	G protein-coupled receptor kinase 5
5	A_23_P23221	GADD45A	0.002036	0.7	0.000982	tumor necrosis factor superfamily, member 4
5	A_23_P115608	ARHGAP21	0.002171	0.72	0.000172	growth arrest and DNA-damage-inducible, alpha
5	A_24_P135444	AMFR	0.002179	0.67	0.000161	Rho GTPase activating protein 21
5	A_24_P118376	UNQ9366	0.002790	0.61	3.09E-05	autocrine motility factor receptor
5	A_23_P124476	CLCN3	0.003450	0.63	0.000154	carcinoembryonic antigen-related cell adhesion molecule 20
5	A_32_P35751		0.003593	0.69	0.000264	chloride channel 3
5	A_32_P103558		0.003653	0.74	0.00498	FLJ37480 fis
5	A_23_P334123	CDA08	0.004356	0.58	7.53E-05	T-cell immunomodulatory protein
5	A_23_P143902	P2RY12	0.005046	0.69	0.00038	purinergic receptor P2Y
5	A_23_P136693		0.005253	0.68	0.0219	DKFZp686D0521
5	A_23_P33947	EFHC2	0.007107	0.66	0.0173	EF-hand domain containing 2
5	A_23_P139486	CDK2API	0.007218	0.62	0.000549	CDK2-associated protein 1

Cluster	ProbeId	Gene Symbol	Average		P-value		Gene Name
			P-value	Pearson	CARGO	LARGO	
5	A_23_P217611	ARMCX3	0.007295	0.67	0.000649	0.082	armadillo repeat containing, X-linked 3
5	A_23_P86424	NCOA4	0.007891	0.52	0.000265	0.235	nuclear receptor coactivator 4
5	A_23_P115375	H3/o	0.007956	0.62	0.03	0.00211	histone H3/o
5	A_23_P91900	SMC4L1	0.008477	0.04	0.00771	0.00932	SMC4 structural maintenance of chromosomes 4-like 1
5	A_23_P422083	DKFZp762O076	0.009500	0.6	0.000586	0.154	transmembrane protein 55A
5	A_23_P69226	LOC55831	0.009851	0.64	0.00239	0.0406	transmembrane protein 111
5	A_23_P59547	NT5C3	0.010860	0.62	0.00819	0.0144	5'-nucleotidase, cytosolic III
5	A_24_P500621		0.012377	0.65	0.00382	0.0401	FLJ23711 fis
5	A_24_P26897	INPP5A	0.012755	0.62	0.0033	0.0493	inositol polyphosphate-5-phosphatase
5	A_23_P11025	ZNF185	0.013107	0.64	0.000872	0.197	zinc finger protein 185
5	A_24_P349560	EIF4E	0.014011	0.54	0.000518	0.379	eukaryotic translation initiation factor 4E
5	A_24_P941699	PCGF5	0.015716	0.56	0.00233	0.106	polycomb group ring finger 5
5	A_24_P147927	EFHC2	0.016470	0.54	0.00508	0.0534	EF-hand domain containing 2
5	A_23_P8763	PTPN12	0.022823	0.6	0.00383	0.136	protein tyrosine phosphatase, non-receptor type 12
5	A_24_P81947	CORO1C	0.024231	0.58	0.00656	0.0895	coronin, actin binding protein, 1C
5	A_23_P371239	CMIP	0.024670	0.46	0.0017	0.358	c-Maf-inducing protein
5	A_23_P135494	CLIC4	0.027543	0.58	0.00875	0.0867	chloride intracellular channel 4
5	A_23_P72643	ADAM9	0.029899	0.54	0.00634	0.141	metallopeptidase domain 9
5	A_24_P503866		0.049292	0.42	0.00968	0.251	CS0DL005YE02
5	A_24_P23411	ARMCX3	0.052866	0.52	0.0137	0.204	armadillo repeat containing, X-linked 3
5	A_24_P633902	ZNF364	0.054895	0.52	0.0123	0.245	zinc finger protein 364
5	A_32_P96134	KIAA0877	0.065465	0.44	0.00698	0.614	KIAA0877
5	A_23_P201376	SSX2IP	0.071789	0.44	0.00901	0.572	synovial sarcoma, X breakpoint 2 interacting protein

Cluster	ProbeId	GeneSymbol	Average		P value		GeneName
			P-value	Pearson	CARGO	LARGO	
5	A 32 P6172		0.075908	0.46	0.215	0.0268	IMAGE:5286843
5	A 24 P27373	PLDN	0.101739	0.42	0.0477	0.217	pallidin homolog
5	A 23 P96041	FLJ22679	0.112988	0.4	0.0202	0.632	FLJ22679
5	A 32 P39384		0.161655	0.09	0.306	0.0854	IMAGE:4823416
6	A 23 P145965	TPST1	0.000010	0.9	3.05E-05	2.97E-06	tyrosylprotein sulfotransferase 1
6	A 23 P33723	CD163	0.000077	0.8	5.09E-05	0.000116	CD163 antigen
6	A 24 P38081	FKBP5	0.000138	0.86	3.82E-05	0.000498	FK506 binding protein 5
6	A 23 P111206	FKBP5	0.000228	0.83	2.71E-05	0.00191	FK506 binding protein 5
6	A 23 P121602	SAP30	0.000244	0.8	0.000484	0.000123	sin3-associated polypeptide
6	A 23 P328729	KLHL8	0.000273	0.82	0.000111	0.000672	kelch-like 8
6	A 23 P104804	ZBTB16	0.000607	0.79	0.00389	9.47E-05	zinc finger and BTB domain containing 16
6	A 23 P99442	FLT3	0.000780	0.79	0.000111	0.00548	fms-related tyrosine kinase 3
6	A 32 P806841	ARL4A	0.001443	0.6	8.40E-05	0.0248	ADP-ribosylation factor-like 4A
6	A 32 P223985	LOC388752	0.001917	0.74	0.00157	0.00234	LOC388752
6	A 24 P32215		0.002288	0.57	7.92E-05	0.0661	ADP-ribosylation factor-like 4B
6	A 23 P145761	ARL4A	0.002289	0.55	0.000139	0.0377	ADP-ribosylation factor-like 4A
6	A 23 P53838	IRS2	0.002515	0.74	0.00111	0.0057	insulin receptor substrate 2
6	A 24 P213296	dJ341D10.1	0.003441	0.75	0.00764	0.00155	dJ341D10.1
6	A 23 P415401	KLF9	0.050850	0.46	0.0117	0.221	Kruppel-like factor 9
7	A 23 P113212	TMEM45A	0.000027	0.82	4.53E-06	0.000165	transmembrane protein 45A
7	A 32 P114020		0.000060	0.85	8.32E-05	4.26E-05	T32824
7	A 32 P29140		0.000061	0.85	0.00182	2.04E-06	AA344632
7	A 32 P130968		0.000137	0.8	4.08E-05	0.000461	IMAGE:4826240
7	A 23 P57658	HRASLS	0.000175	0.74	1.43E-06	0.0214	HRAS-like suppressor
7	A 23 P381714	CA13	0.000259	0.77	8.82E-05	0.000762	carbonic anhydrase XIII
7	A 32 P131449		0.000287	0.74	3.48E-06	0.0237	
7	A 23 P151662	MAX	0.000310	0.81	3.69E-05	0.00261	MYC associated factor X
7	A 23 P17130	MGC13057	0.000319	0.8	7.85E-05	0.0013	hypothetical protein MGC13057
7	A 24 P76675	MFAP3L	0.000336	0.84	7.72E-05	0.00146	microfibrillar-associated protein

Cluster	Probe Id	Gene Symbol	Average		P value		Gene Name
			P value	Pearson	CARGO	LARGO	
							3-like
7	A_23_P331253	XPNPEP1	0.000479	0.76	2.30E-05	0.00998	X-prolyl aminopeptidase 1
7	A_24_P394510	HIST1H2AJ	0.000497	0.72	1.75E-05	0.0141	histone 1, H2aj
7	A_23_P200001	NEXN	0.000587	0.76	0.000104	0.00331	nexilin
7	A_32_P38745		0.000645	0.8	0.000686	0.000606	
7	A_24_P409971	NEXN	0.000784	0.78	5.04E-05	0.0122	nexilin
7	A_24_P363615	MTPN	0.000858	0.72	0.000102	0.00721	myotrophin
7	A_32_P196142		0.000944	0.79	0.00224	0.000398	
7	A_32_P808	KIAA1458	0.000999	0.74	4.66E-05	0.0214	KIAA1458
7	A_32_P79041		0.001349	0.68	0.0346	5.26E-05	IMAGE:6179261
7	A_23_P217938	SPHAR	0.001657	0.73	0.00028	0.0098	S-phase response
7	A_23_P132619	OXTR	0.001859	0.73	0.000898	0.00385	oxytocin receptor
7	A_24_P453819		0.002084	0.71	0.00118	0.00368	IMAGE:30330955
7	A_23_P363344	TPM1	0.002346	0.66	0.000173	0.0318	tropomyosin 1
7	A_23_P365685	LIMS3	0.002380	0.77	0.000759	0.00746	LIM and senescent cell antigen-like domains 3
7	A_24_P148094	LEPROT	0.002416	0.7	0.000111	0.0526	leptin receptor overlapping transcript
7	A_23_P131825	TNNC2	0.002568	0.74	0.00822	0.000802	troponin C type 2
7	A_23_P39202	C19orf33	0.002879	0.7	0.00086	0.00964	chromosome 19 ORF33
7	A_23_P16733	RALB	0.003196	0.65	0.0619	0.000165	v-ral simian leukemia viral oncogene homolog B
7	A_23_P160336	LEFTY1	0.003738	0.74	0.00102	0.0137	left-right determination factor 1
7	A_32_P117908		0.004157	0.64	0.000163	0.106	
7	A_24_P514678		0.004737	0.69	0.00291	0.00771	
7	A_23_P1126	LEPROT	0.004784	0.66	0.0021	0.0109	leptin receptor overlapping transcript
7	A_23_P160582	HT036	0.005785	0.64	0.00651	0.00514	hydroxypyruvate isomerase homolog
7	A_32_P27878		0.006556	0.67	0.00307	0.014	AA399656

Cluster	Probe Id	Gene Symbol	Average		P-value		Gene Name
			P-value	Pearson	CARGO	LARGO	
7	A_23_P93282	HIST1H3J	0.007206	0.65	0.00775	0.0067	histone 1, H3j
7	A_24_P570806		0.008430	0.63	0.00345	0.0206	IMAGE:4814437
7	A_32_P80532		0.008928	0.48	9.84E-05	0.81	BF733908
7	A_24_P35478	PARD3	0.012240	0.6	0.00165	0.0908	par-3 partitioning defective 3 homolog
7	A_23_P38876	LIPE	0.012455	0.32	0.768	0.000202	lipase, hormone-sensitive
7	A_23_P89902	RTN2	0.013112	0.5	0.00033	0.521	reticulon 2
7	A_24_P879895		0.013737	0.62	0.00293	0.0644	IMAGE:3883659
7	A_24_P231104	LEPR	0.015491	0.56	0.00142	0.169	leptin receptor
7	A_24_P524262		0.019790	0.58	0.0611	0.00641	Q80YT0
7	A_23_P38106	SPHK1	0.023166	0.45	0.000905	0.593	sphingosine kinase 1
7	A_23_P137173	TMSNB	0.023397	0.53	0.00238	0.23	thymosin-like 8
7	A_32_P25639	BET3L	0.039122	0.51	0.198	0.00773	FLJ11180 fs
7	A_23_P426663	MITF	0.044996	0.54	0.00703	0.288	microphthalmia-associated transcription factor
7	A_23_P169756	HIPK2	0.045615	0.39	0.549	0.00379	homeodomain interacting protein kinase 2
7	A_23_P92025	CIDEC	0.059978	0.34	0.00363	0.991	cell death-inducing DFFA-like effector c
7	A_32_P181297		0.061180	0.44	0.488	0.00767	CS0DK012YG12
7	A_23_P377214	FLJ32384	0.065383	0.48	0.0217	0.197	hexamethylene bis-acetamide inducible 2
7	A_32_P4433		0.070070	0.46	0.098	0.0501	BU602485
7	A_23_P213050	HPGD	0.089001	0.47	0.0483	0.164	hydroxyprostaglandin dehydrogenase 15-(NAD)
7	A_23_P328740	LOC93082	0.124378	0.42	0.0874	0.177	BC012317
7	A_24_P347447	DAAM1	0.134365	0.39	0.177	0.102	dishevelled associated activator of morphogenesis 1
7	A_23_P54116	DAAM1	0.154932	0.39	0.0934	0.257	dishevelled associated activator of morphogenesis 1

Cluster	Probe Id	Gene Symbol	Average		P value		Gene Name
			P value	Pearson	CARGO	LARGO	
7	A_23_P65674	TMOD3	0.257564	0.32	0.273	0.243	tropomodulin 3
7	A_32_P225135		0.483072	0.23	0.259	0.901	IMAGE:5277859
8	A_23_P46369	RAB13	0.000031	0.83	8.86E-06	0.000106	RAB13
8	A_23_P130961	ELA2	0.000132	0.84	1.56E-05	0.00111	elastase 2
8	A_23_P140384	CTSG	0.000271	0.82	7.50E-05	0.000982	cathepsin G
8	A_23_P86653	PRG1	0.000326	0.75	2.86E-05	0.00372	proteoglycan 1, secretory granule
8	A_23_P141173	MPO	0.000634	0.78	6.51E-05	0.00618	myeloperoxidase
8	A_23_P167005	GPR160	0.001061	0.72	0.00938	0.00012	G protein-coupled receptor 160
8	A_23_P121716	ANXA3	0.001315	0.72	0.00144	0.0012	annexin A3
8	A_23_P326080	DEFA4	0.001467	0.7	0.000333	0.00646	defensin, alpha 4, corticostatin
8	A_24_P347378	ALOX5AP	0.001541	0.71	0.00144	0.00165	arachidonate 5-lipoxygenase-activating protein
8	A_23_P201193	TSPAN2	0.001921	0.71	0.000489	0.00755	tetraspanin 2
8	A_23_P150903	MLSTD1	0.001962	0.71	0.00104	0.0037	male sterility domain containing 1
8	A_23_P131789	BPI	0.002874	0.66	0.00113	0.00731	bactericidal/permeability-increasing protein
8	A_23_P169437	LCN2	0.002906	0.67	0.00364	0.00232	lipocalin 2
8	A_23_P159952	BEX1	0.003754	0.66	0.0052	0.00271	brain expressed, X-linked 1
8	A_23_P69171	SUCNR1	0.004568	0.65	0.00346	0.00603	succinate receptor 1
8	A_23_P71981	ERAL1	0.009085	0.62	0.00907	0.0091	Era G-protein-like 1
9	A_24_P63019	IL1R2	0.000002	0.83	2.00E-06	3.01E-06	interleukin 1 receptor, type II
9	A_23_P60627	ALOX15B	0.000010	0.85	0.000125	8.69E-07	arachidonate 15-lipoxygenase, second type
9	A_23_P4036	HT008	0.000015	0.89	1.98E-05	1.17E-05	testis expressed sequence 2
9	A_23_P117582	JDP2	0.000034	0.84	0.000231	5.06E-06	jun dimerization protein 2
9	A_32_P224094	ZNF143	0.000056	0.79	0.000529	5.91E-06	zinc finger protein 143
9	A_24_P202567	ITPKC	0.000062	0.84	1.65E-05	0.000232	inositol 1,4,5-trisphosphate 3-kinase C
9	A_23_P162668	CPM	0.000082	0.8	0.000296	2.25E-05	carboxypeptidase M

Cluster	ProbeId	Gene Symbol	Average		P value		Gene Name
			P-value	Pearson	CARGO	LARGO	
9	A_23_P255104	LHFPL2	0.000101	0.79	2.49E-06	0.0041	lipoma HMGIC fusion partner-like 2
9	A_23_P155765	HMGB2	0.000113	0.82	2.57E-05	0.000497	high-mobility group box 2
9	A_23_P169529	HRB	0.000139	0.8	5.28E-06	0.00365	HIV-1 Rev binding protein
9	A_23_P116195		0.000162	0.81	0.00481	5.45E-06	Q7PKG0
9	A_23_P11201	GPR34	0.000167	0.84	4.19E-05	0.000666	G protein-coupled receptor 34
9	A_23_P388900	SLC22A15	0.000210	0.81	0.000107	0.000414	solute carrier family 22, member 15
9	A_24_P938352	CPM	0.000269	0.81	0.000125	0.000577	carboxypeptidase M
9	A_23_P423864	PHC2	0.000402	0.76	6.02E-05	0.00268	polyhomeotic-like 2
9	A_23_P138725	MARVELD1	0.000564	0.79	0.000175	0.00182	MARVEL domain containing 1
9	A_24_P269687	TOR1A	0.000586	0.7	5.25E-05	0.00653	torsin family 1, member A
9	A_24_P913115		0.000814	0.77	0.000137	0.00484	CS0DK002YE20
9	A_23_P93562	SESN1	0.001184	0.74	0.000356	0.00394	sestrin 1
9	A_23_P104798	IL18	0.001190	0.78	0.000343	0.00413	interleukin 18
9	A_23_P8640	GPR30	0.001536	0.76	0.00068	0.00347	G protein-coupled receptor 30
9	A_24_P78531	CLEC4E	0.002161	0.77	0.00251	0.00186	C-type lectin domain family 4, member E
9	A_23_P215566	AHR	0.002474	0.75	0.00358	0.00171	aryl hydrocarbon receptor
9	A_23_P415021	DKFZP586A0522	0.003217	0.71	0.00639	0.00162	DKFZP586A0522
9	A_24_P154037	IRS2	0.003607	0.8	0.00215	0.00605	insulin receptor substrate 2
9	A_24_P750164	LOC151438	0.004384	0.71	0.00223	0.00862	FLJ31315 fis
9	A_23_P98085	PTEN	0.004927	0.68	0.00274	0.00886	phosphatase and tensin homolog
9	A_24_P233995	FLJ22390	0.008645	0.69	0.0087	0.00859	MOCO sulphurase C-terminal domain containing 1
10	A_24_P235266	GRB10	0.000044	0.8	2.84E-06	0.000697	growth factor receptor-bound protein 10
10	A_23_P122863	GRB10	0.000207	0.76	5.88E-06	0.0073	growth factor receptor-bound protein 10
10	A_24_P360674	CDKN2B	0.002052	0.69	8.42E-05	0.05	cyclin-dependent kinase inhibitor

Cluster	Probe Id	Gene Symbol	Average		P value		Gene Name
			P value	Pearson	CARGO	LARGO	
10	A 24 P323084	FLJ39421		0.68	0.00106	0.0496	chromosome 17 ORF 55
10	A 23 P502470	IL6ST		0.67	0.00727	0.00739	interleukin 6 signal transducer

Table 2

Gene/ Protein	All Days post-transplant			≤180 days post-transplant				
	Mean R (n=39)	Mean NR (n=65)	Ratio R/NR	p-value*	Mean R (n=28)	Mean NR (n=46)	Ratio R/NR	p-value*
	27.4	23.9	NA	0.01	28.4	22.4	NA	0.0004
IL1R2	34.3	33.6	0.62	0.009	34.4	33.2	0.44	0.0003
PDCD1	32	32.4	1.32	0.03	32	32.4	1.32	0.06
FLT3	32	31.6	0.76	0.11	32.2	31.5	0.62	0.02
PF4	25	24.8	0.87	0.18	25	24.8	0.87	0.27
ITGAM	26.9	26.8	0.93	0.22	27	26.7	0.81	0.07
SEMA7 A	34.3	34.4	1.07	0.31	34.3	34.5	1.15	0.16
RHOA	29.8	29.9	1.07	0.41	29.8	29.9	1.07	0.24
G6b	26.7	26.5	0.87	0.46	26.6	26.5	0.93	0.72
ITGA4	27.6	27.6	1	0.47	27.6	27.7	1.07	0.31
WDR40 A	28.9	28.8	0.93	0.68	28.7	28.8	1.07	0.88
MIR	29.4	29.3	0.93	0.82	29.3	29.3	1	0.85
*Significant values in larger red typeface								

Table 3

Gene/Protein	All times post transplant		<180 da post transplant	
	R (n=38)/NR (n=55)	p-value*	R (n=27)/NR (n=40)	p-value
n	Fold Change		Fold Change	
IL1R1	0.67	0.01	0.55	0.0008
TSC22D3	0.8	0.01	0.72	0.0009
FKBP5	0.85	0.18	0.68	0.007
THBS1	0.73	0.04	0.68	0.03
CD163	0.85	0.2	0.72	0.03
ABCB1	1.1	0.41	1.28	0.07
ANXA1	0.89	0.1	0.86	0.1
IL1B	1.29	0.19	1.45	0.11
EPOR	0.9	0.06	0.91	0.17
DUSP1	0.88	0.39	0.79	0.21
SGK	1.08	0.5	1.16	0.27
TGFB1	0.94	0.19	0.94	0.3
IL7R	1.08	0.54	1.19	0.3
NFKBIA	0.92	0.41	0.9	0.43
NR3C1	1.01	0.76	1.02	0.52
IL4R	0.98	0.75	0.97	0.56
SELP	0.88	0.36	0.93	0.62
IL1RN	0.97	0.73	0.97	0.78
THBS2	0.97	0.74	1.03	0.79
ITGAX	1.02	0.8	0.96	0.86
TNFRSF1	0.94	0.61	1.02	0.89
ADA	1.26	0.002	1.35	0.0008

GZMA	1.19	0.15	1.4	0.01
TRBC1	1.27	0.8	1.5	0.02
FLT3LG	1.16	0.12	1.31	0.03
CD28	1.21	0.12	1.33	0.08
CD8A	1.15	0.37	1.32	0.1
PDCD1L	1.2	0.6	1.21	0.12
CTLA4	1.19	0.17	1.23	0.18
CD274	1.08	0.38	1.15	0.2
CD4	1.01	0.87	1.08	0.35
NFKB1	1.09	0.03	1.1	0.02
TNF	1.21	0.06	1.32	0.03

EXAMPLES

Example 1 Study Objectives and Subjects

[0054] Nucleic acid technologies were used to produce gene expression profiles for PBMC samples from subjects who had been treated with various dosages of steroid and were enrolled in the Cardiac Allograft Rejection Gene Expression Observational (CARGO) and the Lung Allograft Rejection Gene Expression Observational (LARGO) studies. All studies were approved by local Institutional Review Boards.

[0055] The CARGO study was initiated in 2001 to study gene expression in blood samples as a means for managing transplant rejection in cardiac patients. The eight transplant centers contributing to the studies handle more than 20% of cardiac transplants. The LARGO study was initiated in 2004 to collect blood samples and clinical data, including the results from TBB from lung transplant subjects, at fourteen centers in five different countries.

[0056] Microarrays as described in Example 10 were used to study gene expression in 95 samples from LARGO subjects being treated with 5-40 mg of steroid, 68 samples from CARGO subjects being treated with 1-100 mg of steroid, and 56 samples from CARGO or LARGO subjects being treated with 0-50 mg of steroid for CMV infection.

[0057] RT-PCR was used in exemplary and pathways studies with PBMC samples from CARGO subjects between 30 days and 12 months post-transplant whose transplants were graded as rejection or non-rejection. The principle inclusion criteria were: a) clinically stable defined as absence of signs or symptoms of acute cardiac allograft rejection, b) histologically stable defined as current EMB indicating non-rejection, c) absence of cardiac dysfunction by invasive hemodynamics and/or echocardiogram, and d) absence of ISHLT (International Society for Heart and Lung Transplant) $\geq 3A$ rejection, graft dysfunction, or administration of rejection therapy within 30 days prior to enrollment. The demographic and treatment characteristics of the cardiac transplant subjects are shown in the following Table 4.

Table 4

Groups—No Subjects	Subjects—all days post transplant			Subjects ≤ 180 days post transplant		
	R=39	NR=65	p-value	R=28	NR=46	p-value
Median Age (Range)	60 (25-68)	59 (8-76)	0.58	59 (25-68)	59 (8-76)	0.73
Sex—Male (%)	32 (82.1)	54 (83.1)	1	22 (78.6)	41 (53.6)	0.31

Race—No (%)			0.33		0.025
White	23 (59.0)	47 (72.3)		15 (53.6)	38 (82.6)
Black	10 (25.6)	10 (15.6)		8 (28.6)	5 (10.9)
Other	6 (15.4)	8 (12.1)		5 (17.8)	3 (6.5)
Immunosuppression Regimen — No (%)			0.32		0.29
Cyclosporine/Mycophenolate	20 (51.3)	37 (56.9)		15 (53.6)	28 (60.9)
Cyclosporine/Sirolimus	1 (2.6)	2 (3.1)		1 (3.6)	2 (4.3)
Tacrolimus/Mycophenolate	10 (25.6)	19 (29.2)		6 (21.4)	12 (26.1)
Tacrolimus/Sirolimus	6 (15.4)	3 (4.6)		5 (17.9)	2 (4.3)
Other	2 (5.1)	4 (6.2)		1 (3.6)	2 (4.3)
Median Dose (Range)					
Index Sample	10 (2-30)	10 (1-60)	0.62	13.25 (2-30)	12.5 (1-60)
R/NR Sample	7.5 (1-25)	7.5 (2-20)	0.8	10 (2-25)	10 (2.5-20)
Post-recovery Sample	10 (1-80)	6 (1-20)	0.003	10 (2-80)	7.5 (2-20)
Days Post-Transplant — Median (Range)					
Index Sample	138 (32-491)	133 (33-317)	0.3	93 (32-180)	83 (33-177)
R/NR Sample	180 (53-565)	166 (56-342)	0.33	130 (53-240)	124 (56-242)
Post-recovery Sample	189 (62-579)	228 (70-471)	0.56	155 (62-249)	152 (70-304)
Days from Index to R/NR	35 (14-77)	34 (14-76)	0.99	32 (14-63)	31 (14-70)
ISHLT Biopsy — No (%)			0.0006		0.008
Grade 0	12 (30.8)	43 (66.2)		9 (32.1)	30 (65.2)
Grade 1A	27 (69.2)	22 (34.4)		19 (67.9)	16 (34.8)

[0058] Column 1 of the table characterizes the subjects, immunosuppression regimen, days post-transplant and ISHLT grades. Columns 2, 3 and 4 show the data for rejection (R) and non-rejection (NR) subjects and p-values for characteristics all days post-transplant. Columns 4, 5, and 6 show the data for rejection (R) and non-rejection (NR) subjects and p-values for characteristics <180 days post transplant.

[0059] Subjects in both the R and NR groups were on standard steroid weaning protocols with no significant difference ($p=0.75$) in steroid dose. A two-tailed independent t-test or a Fisher Exact test was used to compare quantitative characteristics, and a Wald (Mann Whitney) test was used to compare categorical characteristics. There was no significant difference in the distribution of characteristics between groups except that ISHLT 1A biopsies and African-Americans were more prevalent in the R group.

Example 2 Sample Collection, Transplant Protocol, and Immunosuppressive Therapy

[0060] A blood sample was collected from each subject at each clinical encounter, and clinical data including results of EMB or TBB, immunosuppressive regime, laboratory data, and

clinical complications were obtained. Samples were processed as described in Example 8.

[0061] Standard cardiac transplant center protocols generally require invasive EMBs to be performed weekly in the first 30 days post transplant (4 biopsies), every two weeks between 31-90 days post transplant (4 biopsies), every 4 weeks between 91-180 days post transplant (3 biopsies), and every 8 weeks between 181-365 days post transplant (3 biopsies). Histology was graded by a local pathologist and two or three pathologists blinded to subject data and outcomes. Agreement of at least two of the pathologists was required to diagnose ISHLT $\geq 3A$ rejection, and agreement of three pathologists was required for ISHLT 0/1A non-rejection.

[0062] Standard lung transplant center protocols generally require at least six invasive TBBs during the first six months post transplant. These tissue samples are examined by at least three pathologists for signs of rejection and rated on a five point ISHLT scale of increasing severity based on the extent of perivascular inflammation, A0=normal lung tissue, A1=minimal, A2=mild, A3=moderate, and A4=severe rejection. A TBB rated $\geq A2$ generally requires therapeutic intervention.

[0063] All subjects received center-specific immunosuppressive therapy consisting of cyclosporine or tacrolimus in combination with either mycophenolate mofetil or sirolimus and corticosteroids. The cardiac rejection group (R) had 39 subjects who progressed to acute cellular rejection within 12 weeks. The control group (NR) had 65 subjects who remained rejection-free and were matched with subjects in the rejection group by demographic characteristics, time post-transplant, and immunosuppressive therapy.

Example 3 Steroid Modulated Nucleic Acids and Their Expression

[0064] Steroid modulated genes are described in the clusters of Table 1, in the diagnostic set of genes of Table 5, in the pathways genes of Table 3, and among the sequences listed in the published applications and patents incorporated by reference herein in their entirety and shown in the table below.

Table 5

Title	Application No; Filing Date	Patent/Publication No
Methods And Compositions For Diagnosing And Monitoring Autoimmune And Chronic Inflammatory Diseases	USSN 10/131,827; 4/24/2002 PCT/US03/13015; 4/24/2003	USPN 6,905,827 WO03/090694

Methods And Compositions For Diagnosing And Monitoring Transplant Rejection	USSN 10/325,899; 12/20/2002 PCT/US03/129456	US2003/123086 WO04/042346
Leukocyte Expression Profiling	PCT/US01/47856; 10/22/2001	WO02/057414

[0065] The steroid modulated genes were identified using at least one statistical method on nucleic acid expression from the microarray study as described in Example 4 and RT-PCR studies as described in Example 5. Primers and probe sets for use in a diagnostic set for detecting genes modulated by steroids can be generated as described in Examples 11 and 12.

Example 4 Microarray Study and Results

[0066] Protocols used with the microarrays are described in Examples 9 and 10. For the microarray studies, the manufacturer's software was used to download microarray data. To be included in the analysis, a probe had to be flagged as present (versus marginal or absent) and have a signal of at least 100 for at least 80% of the arrays.

[0067] Nucleic acids expressed on Human Genome CGH 44A microarrays (Agilent Technologies, Palo Alto CA) that correlated with steroid treatment were identified separately in the samples from the CARGO and LARGO projects. Feature Extraction and GeneSpring software (Agilent Technologies) were used to download microarray data. As shown in the first table in Example 1, the initial filtering flagged 28,997 out of 41,000 probes. Signals were normalized to the median expression of each chip to achieve chip-to-chip comparability.

[0068] K-means clustering was applied to the expression of 28,997 nucleic acids in 219 samples as shown in the table below. The parameters for clustering were the number of clusters (20), number of iterations (400), and similarity measure (p-value, Pearson correlation). In one alternative, similarity measure can be a t-test.

[0069] In the initial analysis, nucleic acid expression converged after 147 iterations. Using a p-value <0.01, CARGO samples showed expression in 3,604 genes; LARGO samples, in 699 genes. The CARGO and LARGO samples had 278 expressed nucleic acids in common, and cluster 14 (highlighted) was found to be highly enriched in steroid modulated (SM) genes (62.9%), with another 24.7% whose expression correlated with steroid dose (CSD).

Table 6

Cluster	No. Genes	No. SM Genes	% SM Genes	% of CSD Genes
1	1904	2	0.7	0.1
2	1562	2	0.7	0.1
3	2218	2	0.7	0.1
4	3236	2	0.7	0.1
5	2212	5	1.8	0.2
6	1305	1	0.4	0.1
7	2024	1	0.4	0
8	803	0	0	0
9	1174	2	0.7	0.2
10	2059	24	8.6	1.2
11	975	1	0.4	0.1
12	1219	2	0.7	0.2
13	336	0	0	0
14	709	175	62.9	24.7
15	304	20	7.2	6.6
16	1015	3	1.1	0.3
17	3303	6	2.2	0.2
18	515	3	1.1	0.6
19	981	0	0	0
20	1143	27	9.7	2.4
Total	28997	278	100	37.2

[0070] Column one of Table 6 shows the cluster number; column two, the number of genes in that cluster; column 3, the number of steroid modulated genes; column four, the percent of steroid modulated genes; and column five, the percent of genes correlated with steroid dose.

[0071] Candidate steroid modulated nucleic acids (709 genes from cluster 14 and 278 steroid dose correlated genes) were subjected to additional rounds of K-means clustering. The parameters were number of clusters (40), number of iterations (100), and similarity measure (p-value, Pearson correlation). After each round, any cluster containing zero or one steroid modulated nucleic acid was eliminated. Clusters containing two or more steroid modulated nucleic acids were combined for next round of clustering. After four rounds of K-means clustering, 518 genes were in clusters that contained two or more steroid modulated nucleic acids and 262 (50.5%) were nucleic acids whose expression were correlated with steroid dose (data not shown). These 518 genes were subjected to further rounds of clustering with the parameters: number of clusters (10), number of iterations (100), similarity measure (p-value, Pearson correlation). As shown in the table below, all genes had converged into ten clusters after 14 iterations. The 518 steroid modulated genes are described in their respective clusters in Table 1.

Table 7

Cluster	No. of SM genes	No. CSD Genes
1	116	46
2	95	55
3	73	21
4	45	40
5	67	20
6	15	11
7	58	22
8	16	16
9	28	28
10	5	3
Total	518	262

[0072] Column one of Table 7 shows the cluster number; column two, the number of genes; and column three, the number of genes correlated with steroid dose (CSD).

Example 5 RT-PCR Studies and Results

[0073] An exemplary RT-PCR study demonstrated the utility of steroid modulated nucleic acids and proteins in diagnosing and monitoring steroid responsiveness. Genes were chosen for the diagnostic set, and nucleic acid expression was reported as threshold cycle (CT) as measured using RT-PCR. The ratios of expression are calculated from the Ct values as $2^{(Ct(\text{Control}) - Ct(\text{Rejection}))}$

[0074] Gene expression was processed into a single score using voting, logistic regression or linear algorithms as detailed in Examples 1-3 of USSN 11/433,191 and in Example 5 of USPN 6,905,827, both incorporated by reference herein in their entirety. The diagnostic set of the 20 genes (11 informative, six normalization, three control) contained probes that were designed and tested as described in Examples 11 and 12, and RT-PCR, as described in Example 13, was conducted in triplicate RT-PCR reactions on samples from subjects on standard weaning protocols.

[0075] Of 104 index subjects, longitudinal gene expression profiles including post rejection and matched post non-rejection samples were available for 34 R subjects and 56 matched NR subjects at similar time points. The findings of the index study were extended to include samples and expression from an additional 192 consecutive subject encounters satisfying the inclusion criteria stated above. This set included samples from 118 new subjects and from 74 previous subjects and was used to estimate the prevalence of non-rejection in any 12 week period following sampling.

[0076] Longitudinal changes in expression from the index group were compared to corresponding scores for the larger group of 192 using repeated measure ANOVA. The probability that the transplant would not be rejected (negative predictive value) was calculated using EMB, rejection and non-rejection data. The Wald test was used with multivariate analysis to determine if, after controlling for clinical variables, the gene expression score remained a significant predictor of rejection.

[0077] Gene expression score, as calculated using a prediction algorithm, was found to be an independent predictor of future rejection at $p=0.0266$ when the clinical variables of recipient age, gender and race, panel reactive antibody, CMV serology status, and immunosuppression regimen (Wald test) were included. In fact, independent predictive value at $p=0.015$, was further enhanced in subjects ≤ 180 days post-transplant.

[0078] Table 2 showed the p-value, as calculated using a t-test, for gene expression score and subject nucleic acid expression for 104 index samples, and for the subset of 74 samples ≤ 180 days post-transplant. Several of the individual genes shown in Table 2 showed differential expression associated with acute transplant rejection. Expression of IL1R2 decreased significantly ($p=0.009$, 1.6 fold) and PDCD1, increased significantly ($p=0.032$, 1.3 fold). In addition, IL1R2 ($p<0.001$) and FLT3 ($p=0.024$) demonstrated greater significance during the ≤ 180 day period and significant decreases in expression (2.3 and 1.6 fold, respectively) in subjects who progressed to rejection. During acute rejection, erythropoiesis genes, MIR and WDR40A, were up-regulated (both $p=0.02$), and FLT3 was down-regulated ($p=0.03$). The overall score was also significant using a Wilcoxon test for all subjects who progressed to rejection, $p=0.011$, and for those who did not progress, $p<0.001$. Those subjects who showed evidence of incipient rejection were placed immediately on anti-rejection therapy and subsequently showed a significant decrease in gene expression score ($p<0.01$).

[0079] The first RT-PCR study using a diagnostic set corresponding to the genes shown in Table 2 concluded: a) treatment of rejection with high dose steroids led to a statistically significant change in expression, b) low expression scores or a low value derived from evaluating expression scores with a prediction algorithm identified a group of subjects at very low risk for current and future rejection, and c) expression can be used to stratify subjects as to their risk of future rejection and lead to reduced number of cardiac biopsies.

[0080] The second RT-PCR study used PMBC samples from CARGO subjects and 33 nucleic acids/genes expressed in steroid modulated pathways. Analyses were based on all samples for which mRNA was available, 93 of 104 subjects in the all times post transplant group and 67 of 74 subjects in the ≤ 180 days post transplant group. Most of the nucleic acids came from the IL-1 and PDCD1 pathways and nucleic acids induced and expressed in T cells.

[0081] Table 3 shows the 33 genes grouped as to pathway, T cell associated, and other (TNF and NFKB1) and presented according to p-value within the group. Differential expression of the genes is presented as fold change calculated as $2^{(\text{mean control Ct} - \text{mean rejection Ct})}$. Genes whose mRNA levels demonstrated a fold change >1 were up-regulated (increased) in subjects with rejection while those with a fold change <1 were down-regulated (decreased). P-value was based on t-test, and similar significance was obtained using the Mann-Whitney non-parametric test.

[0082] Using a p-value <0.05 , five of the additional 33 genes tested supported the algorithm's steroid modulated constituents (IL1R2 and FLT3) while six, supported T-cell activation (PDCD1). Specifically, IL1R1, TSC22D3, FKBP5, THBS1 and CD163 showed significantly reduced expression; and ADA, GZMA, TRBC1, NFKB1, TNF and FLT3LG, significantly increased expression. Thus the methods of the invention and diagnostic sets of genes including but not limited to ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3 and selected from Tables 1-3 can be used for determining, diagnosing, evaluating, monitoring, or predicting disease activity, non-rejection, rejection, status of a transplant or of an immune disorder, steroid responsiveness, and treatment plan of a subject with a transplant or immune disorder.

[0083] Informative nucleic acids from the RT-PCR studies are listed in the table below as referenced to sequences in USPN 6,905,827 or GenBank.

GENE	SEQ ID NOs in USPN 6,905,827
CD163	3857
FKBP5	6299

FLT3	See GenBank sequence NM_004119
IL1R2	4685
ITGAM	1981, 62
THB1	4109, 264

Example 6 Prediction of Rejection or Non-Rejection

[0084] Quartile analysis was applied to the exemplary RT-PCR data for 74 subjects ≤ 180 days post transplant. Subjects in the lowest quartile had expression scores ≤ 20 , and no subjects progressed to rejection in the subsequent 12 weeks (n=19). Subjects in the top quartile had expression scores ≥ 30 , and 58% of these subjects had rejection episodes (n=19) within 12 weeks of histological stability.

[0085] When this analysis was extended to the larger group of 192 representative consecutive samples, the incidence of subjects with expression scores ≤ 20 were 33% of samples ≤ 180 days post-transplant, and 98.9% of these remained rejection-free during the ensuing 12 weeks. Since the predictive value did not differ significantly by segmental time periods post transplant (30-60; 61-90; 90-180 days), a clinician can order 2-5 fewer EMBs for a subject with a low risk of rejection during the subsequent 12 weeks.

Example 7 Statistical Methods

[0086] The steroid modulated nucleic acids shown in the tables herein were identified in samples from subjects to whom steroids had been administered using at least one statistical method selected from various classification and prediction algorithms, software and programs. These methods include, but are not limited to, analysis of variance, classification and regression trees (Brieman et al. (1984) Classification and Regression Trees, Wadsworth, Belmont CA), cluster analysis including K-means clustering (MacQueen (1967) *Proceedings of 5th Berkeley Symposium on Mathematical Statistics and Probability*, University of California Press 1:281-297), Fisher Exact test, linear discriminatory analysis, logistic regression (Agresti (1996) *An Introduction to Categorical Data Analysis*. John Wiley and Sons Inc), multiple additive regression trees (Friedman (2002) Stanford University, Stanford CA), Mann-Whitney test, multivariate analysis, nearest shrunken centroids classifier (Tibshirani et al. (2002) PNAS 99:6567-6572), significance analysis of microarrays (Tusher et al. (2001) PNAS 98:5116-5121), one and two tailed T-tests, Wald test (Wald (1943) Trans Am Math Soc 54:426-482), Wilcoxon's signed ranks test, quartile analysis, and the like. Many of the above methods can be performed using SAS (SAS Institute, Cary NC) or Statistica (Statsoft, Tulsa OK). As noted in

Example 1, the statistical methods applied to expression in order to chose a diagnostic set of nucleic acids or proteins are fully described in the Examples 1-3 of USSN 11/433,191 and in Example 16 of USPN 6,905,827, both incorporated by reference herein in their entirety.

Example 8 Preparation of Blood Samples, RNA Isolation from Lysate

[0087] Peripheral blood mononuclear cells (PBMC) were isolated from 8 mL venous blood using a VACUTAINER CPT tube (BD Biosciences (BD), San Jose CA) containing the anticoagulant sodium citrate, Ficoll Hypaque density fluid, and a thixotropic polyester gel. After the blood and tube components were mixed by inverting the tube 5-10 times, the tube was centrifuged, and mononuclear cells were collected from the fluid above the barrier layer. Approximately 2 mls of mononuclear cell suspension were transferred to a microfuge tube and centrifuged for 3 min at 16,000 rpm to pellet the cells. The pellet was resuspended and pipetted up and down in 1.8 ml of RLT lysis buffer (Qiagen, Chatsworth CA). Cell lysate was frozen and stored at -80EC until total RNA was isolated.

[0088] After adding 5 ml of chloroform to the thawed lysate, the samples were vortexed and incubated at room temperature for 3 min. The aqueous layer was transferred to a new tube and purified using the RNeasy kit (Qiagen) according to the manufacturer's protocol. Isolated RNA was treated with DNase on a QIASHREDDER column (Qiagen) and purified RNA was eluted in 50 µl of water. RNA purity was checked using the 2100 bioanalyzer and RNA 6000 microfluidics chips (Agilent Technologies, Palo Alto CA).

[0089] In the alternative, blood samples were collected in PAXgene Blood RNA tubes (Qiagen, Valencia CA and total RNA was purified using the PAXgene Blood RNA kit (Qiagen).

Example 9 cDNA Synthesis

[0090] cDNA was synthesized from purified RNA using reverse transcription with OLIGO-dT primers/random hexamers (Invitrogen, Carlsbad CA) at a final concentration of 0.5 ng/µl and 3 ng/µ, respectively. For the first strand reaction, 0.5 µg of mononuclear RNA and 1 µl of the OLIGO-dT/random hexamers (Invitrogen) were added to water in a reaction tube to a final volume of 11.5 µl. The tube was incubated at 70°C for 10 min, chilled on ice, centrifuged, and 88.5 µl of first strand buffer mix (Invitrogen) was added to the tube.

[0091] The first strand buffer mix contained 1 x first strand buffer, 10 mM DTT (Invitrogen),

0.5 mM dATP (New England Biolabs (NEB), Beverly MA), 0.5 mM dGTP (NEB), 0.5 mM dTTP (NEB), 0.5 mM dCTP (NEB), 200 U of SUPERScript RNase H reverse transcriptase (Invitrogen), and 18 U of RNAGuard inhibitor (GE Healthcare (GEH), Piscataway NJ). After the reaction was incubated at 42°C for 90 min, the enzyme was heat-inactivated at 70°C for 15 min. After adding 2 U of RNase H (NEB) to the reaction tube, it was incubated at 37°C for 20 min.

[0092] For second strand synthesis, 40 U of *E. coli* DNA polymerase (Invitrogen) and 2 U RNaseH (Invitrogen) were added to the previous reaction to bring the final volume to 150 µl. Salts and nucleotides were added to a final concentration of 20 mM Tris-HCl (pH 7.0; Fisher Scientific, Pittsburgh PA), 90 mM KCl (Teknova, Half Moon Bay CA), 4.6 mM MgCl₂ (Teknova), 10 mM (NH₄)₂SO₄ (Fisher Scientific), 1 x second strand buffer (Invitrogen), 0.266 mM dGTP, 0.266 mM dATP, 0.266 mM dTTP, and 0.266 mM dCTP.

[0093] After second strand synthesis for 150 min at 16°C, the cDNA was purified away from the enzymes, dNTPs, and buffers using phenol-chloroform extraction followed by ethanol precipitation in the presence of glycogen. Alternatively, the cDNA was purified on a QIAQUICK silica-gel column (Qiagen) followed by ethanol precipitation in the presence of glycogen. The cDNA was centrifuged at >10,000 x g for 30 min. After the supernatant was aspirated, the pellet was washed with 150 µl of 70% ethanol and centrifuged. Following centrifugation, the supernatant was removed, and residual ethanol evaporated.

Example 10 Arrays

[0094] Arrays were used to identify steroid modulated genes in gene expression profiles from CARGO and LARGO subjects treated with steroids. In basic format, an array contains reagents specific for at least two nucleic acids or proteins, one that binds to a gene product of the invention and one that binds to a control gene product.

[0095] Nucleic Acid Arrays

[0096] Human Genome CGH 44A microarrays (Agilent Technologies) were used to determine differential expression. These Cy3/Cy5 chips contained 41,675 probes (60-mers) that represented most the genes found in REFSEQ database (NCBI); additional genes on the chip represented various controls. The chips were run as recommended by the manufacturer and scanned using an Agilent DNA microarray scanner. The data was extracted using Feature Extraction v 7.5 software (Agilent Technologies).

[0097] In the alternative, Affymetrix U133A Human GeneChips (Affymetrix, Santa Clara CA) with probe sets representing about 14,500 full length genes and 22,000 features were used according to the manuals and product inserts supplied by the manufacturer. Affymetrix Microarray Suite (MAS) v 5.0 software was used to generate expression values for each gene. To correct for slight differences in overall chip hybridization intensity and allow for comparison between samples, each chip was scaled to an overall intensity of 1500.

[0098] In another alternative, a low density array containing amplicons produced using probe sets for the nucleic acids selected from Tables 1-3 are harvested from PCR reactions, purified using Sephacryl-400 beads (GEH) and arrayed on a membrane. The membrane is UV irradiated, washed in 0.2% SDS at room temperature and rinsed three times in distilled water. Non-specific binding sites on the array are blocked by incubation in 0.2% casein in PBS for 30 min at 60°C, and the arrays are washed in 0.2% SDS and rinsed in distilled water prior to hybridization.

[0099] cDNAs are prepared from subject blood samples; diluted to a concentration of 40-50 ng in 45 µl TE buffer, denatured by heating to 100°C for five min, and briefly centrifuged. The denatured cDNA is prepared using the Amersham CYSCRIBE first strand cDNA labeling kit (GEH) according to the manufacturer's instructions. The labeling reaction is stopped by adding 5 µl of 0.2M EDTA, and probe is purified from unincorporated nucleotides using a GFX Purification kit (GEH). The purified probe is heated to 100°C for five min, cooled for two min on ice, and used in membrane-based hybridizations as described below.

[0100] Membranes are pre-hybridized in hybridization solution containing 1% Sarkosyl and 1 x high phosphate buffer (0.5 M NaCl, 0.1 M Na₂HPO₄, 5 mM EDTA, pH 7) at 55°C for two hr. The probe is diluted in 15 ml fresh hybridization solution and added to the membrane. The membrane is hybridized with the probe at 55°C for 16 hr. Following hybridization, the membrane is washed once for 15 min at 25°C in 1 mM Tris (pH 8.0) and 1% Sarkosyl and four times for 15 min each at 25°C in 1 mM Tris (pH 8.0). To detect hybridization complexes, the membrane is exposed to x-ray film (Eastman Kodak) overnight at -70°C, developed, and examined visually or quantified using a scintillation counter (BeckmanCoulter, Fullerton CA).

Antibody arrays

[0101] Monoclonal antibodies are immobilized on a membrane, slide or dipstick or added to the wells of an ELISA plate using methods well known in the art. The array is incubated in the presence of serum or cell lysate until protein:antibody complexes are formed. The proteins

encoded by genes or their splice variants are identified by the known position and labeling of the antibody that binds an epitope of that protein on the array. Quantification is normalized using the antibody:protein complex of various controls.

Example 11 Designing and Selecting Primers

[0102] Primers and probe sets were designed for the steroid modulated, normalization, and control genes using the PRIMER3 program (Whitehead Research Institute (WRI), Cambridge MA). Default values were used for all parameters but melting temperature (T_m). T_m was set between 71.7 and 73.7°C; amplicon size, between 50 and 150 bases in length (optimum, about 100 bases); and primers or probes were allowed to be 36 nucleotides in length. Salt concentration, a critical parameter affecting the T_m of the probes and primers, was used at the default concentration, 50 mM.

[0103] The C source code for the PRIMER3 program was downloaded and compiled for use on machines running the Windows operating system (Microsoft, Redmond WA). To generate a number of potential primers, the program was run in batch mode from the command line using an input file that contained the sequences and the parameters for primer design. The first step was masked out repetitive sequences in the mRNA using the REPEATMASKER program (Institute for Systems Biology, University of Washington, Seattle WA). The second step masked out all known SNPs with allelic heterozygosity higher than 1% as annotated in the SNP database at NCBI (Bethesda MD). The masked sequence was submitted to PRIMER3 using the parameters above, and the top pairs of primers were selected. Alternatively, the Primer3 program was used on the MIT website (Massachusetts Institute of Technology, Cambridge MA) to examine a specific region of the mRNA of a gene.

[0104] In the alternative, primer design software such as the web-based ProbeFinder software (Roche Diagnostics, Indianapolis IN), or provided by other suppliers of oligonucleotides, can be used to design primers and probes sets of the invention. The two step design process requires the name of the target organism and a sequence, gene name, or transcript ID number. The software will identify the Universal ProbeLibrary probes that will detect the nucleic acid. Primers were ordered from Roche Diagnostics, Integrated DNA Technologies (Coralville IA), or a similar commercial source.

Example 12 Testing of Primers and Probe Sets

[0105] Control genes: Experimental variation was monitored by adding one or more control genes to each array. β -actin, β -GUS, 18s ribosomal subunit, GAPDH, and β 2-microglobulin were selected for low variability between samples and high expression across samples.

[0106] Primer Testing: Primers were tested at least once to see whether they produced an amplicon of the correct size and to determine their efficiency in a set of RT-PCR reactions using 5 serial dilutions of cDNA in water (1:10, 1:20, 1:40, 1:80, and 1:160). Each primer pair was tested on cDNA made from mononuclear cell RNA. The PCR reaction contained 1 x RealTime-PCR buffer (Ambion, Austin TX), 2 mM $MgCl_2$ (ABI), 0.2 mM dATP (NEB), 0.2 mM dTTP (NEB), 0.2 mM dCTP (NEB), 0.2 mM dGTP (NEB), 0.625 U AMPLITAQ Gold enzyme (ABI), 0.3 μ M of each primer to be used (Sigma Genosys, The Woodlands TX), 5 μ l of the reverse transcription reaction, and water added to a final volume of 19 μ l.

[0107] Following 40 cycles of PCR, 10 μ l of each PCR product was combined with Sybr Green dye at a final dilution of 1:72,000. Melt curves for each product were determined on a PRISM 7900HT Sequence detection system (ABI), and primer pairs yielding a product with one clean peak were chosen for further analysis. One μ l of product from each probe set assay was examined by agarose gel electrophoresis or using a DNA 1000 chip kit and an Agilent 2100 bioanalyzer (Agilent Technologies). From primer design and the genomic sequence, the expected size of the amplicon was known. Only primer pairs showing amplification of the single desired product, and minimal amplification of contaminants, were used in assays.

Example 13 RT-PCR Assays and Analysis

[0108] CARGO: Ten μ l RT-PCR reactions were performed to evaluate expression in the CARGO samples. TAQMAN Universal PCR Master mix (ABI) was aliquoted into light tight tubes, one for each gene. The primer pair for each gene was added to the tube of PCR master mix labeled for that gene. A FAM/TAMRA dual labeled TAQMAN probe (Biosearch Technologies, Novato CA) was added to each tube. Alternatively, different combinations of commercially available fluorescent reporter dyes and quenchers were used such that the absorption wavelength for the quencher matches the emission wavelength for the reporter. In the alternative, Universal ProbeLibrary probes (LNAs; Roche Diagnostics) were substituted for TAQMAN probes.

[0109] Assays and Analysis: Each sample was dispensed into triplicate wells of a 384 well plate (ABI) for each primer pair. PCR reactions were run on the PRISM 7900HT Sequence Detection system (ABI) with the following conditions: 10 min at 95°C; 40 cycles of 95°C for 15 sec, 60°C for 1 min. Sequence detection system v2.0 software (ABI) was used to analyze the fluorescent signal from each reaction. RT-PCR amplification product was measured as CT during the PCR reaction to observe amplification before any reagent became rate limiting. Threshold was set to a point where all of the reactions were in their linear phase of amplification. A lower CT indicated a higher amount of starting material (greater expression in the sample) since an earlier cycle number meant the threshold was crossed more quickly. A CT of less than 30 based on appropriate cDNA dilutions provided linear results for the blood samples from CARGO subjects. In the alternative, other technologies can be used to measure PCR product. Molecular beacons (Invitrogen) use FRET technology and disposable, microfluidic chip (Thermal Gradient, Pittsford NY) employ silicon wafers to performed 30 cycle PCR in 4.4 min.

Example 14 Labeling Moieties

[0110] Labeling moieties can be used for detection of an antibody, nucleic acid or protein in any of the assays or diagnostic kits described herein. These labeling moieties include fluorescent, chemiluminescent, or chromogenic agents, cofactors, enzymes, inhibitors, magnetic particles, radionuclides, reporters/quenchers, substrates and the like that can be attached to or incorporated into the antibody, nucleic acid or protein. Visible labels and dyes include but are not limited to anthocyanins, avidin-biotin, β glucuronidase, biotin, BIODIPY, Coomassie blue, Cy3 and Cy5, 4,6-diamidino-2-phenylindole (DAPI), digoxigenin, ethidium bromide, FAM/TAMRA, FITC, fluorescein, gold, green fluorescent protein, horseradish peroxidase, lissamine, luciferase, phycoerythrin, reporter/quencher pairs (HEX/TAMRA, JOE/TAMRA, ROX/BHQ2, TAMRA/BHQ2, TET/BHQ1, VIC/BHQ1, and the like), rhodamine, spyro red, silver, streptavidin, and the like. Radioactive markers include radioactive forms of hydrogen, iodine, phosphorous, sulfur, and the like. They can be added to a primer or probe or to an antibody using standard protocols well known in the art and described in the specific nucleic acid and protein technologies described in Examples 9-14 and 16-17, respectively.

Example 15 Protein Expression

[0111] Adapter sequences for subcloning are added at either end of a coding region specific to a gene or a portion thereof and amplified using PCR. An epitope or affinity tag (6 x his) or

sequences for secretion from a cell can be added to the adapter sequence to facilitate purification and/or detection of the protein. The amplified cDNA is inserted into a shuttle or expression vector that can replicate in bacteria, insect, yeast, plant, or mammalian cells. Such vectors typically contain a promoter that operably links to the coding region, replication start sites, and antibiotic resistance or metabolite selection sequences.

[0112] The expression vector can be used in an in vitro translation system or to transfect cells. For example, Spodoptera frugiperda (Sf9) insect cells are infected with recombinant Autographica californica nuclear polyhedrosis virus (baculovirus). The polyhedrin gene is replaced with the cDNA by homologous recombination, and the polyhedrin promoter drives transcription. The protein is synthesized as a fusion protein with an affinity tag that enables purification.

[0113] Clones of transformed cells are analyzed to ensure that the inserted sequence is expressed. Once expression is verified, the cells are grown under selective conditions; and the protein is isolated from cells, or if secreted, from the growth media using chromatography, size exclusion chromatography, immunoaffinity chromatography, or other methods including cell fractionation, ion exchange, or selective precipitation.

[0114] The isolated and purified protein is then used as a reagent on an array or as an antigen to produce specific antibodies.

Example 16 Antibody Production and Testing

[0115] If antibodies are to be used as reagents, the sequence of the gene or splice variant is analyzed to determine regions of high immunogenicity (LASERGENE software; DNASTAR, Madison WI), and an appropriate oligopeptide is synthesized and conjugated to keyhole limpet hemocyanin (KLH; Sigma-Aldrich, St Louis MO).

Immunization

[0116] Rabbits are injected with the oligopeptide-KLH complexes in complete Freund's adjuvant, and the resulting antisera is tested for specific recognition of the protein or fragments thereof. Antisera that react positively with the protein are affinity purified on a column containing beaded agarose resin to which the synthetic oligopeptide has been conjugated (SULFOLINK kit; Pierce Chemical, Rockford IL). The column is equilibrated using 12 ml IMMUNOPURE Gentle Binding buffer (Pierce Chemical). Three ml of rabbit antisera is

combined with one ml of binding buffer and poured into the column. The column is capped (on the top and bottom), and antisera is allowed to bind with the oligopeptide by gentle shaking at room temperature for 30 min. The column is allowed to settle for 30 min, drained by gravity flow, and washed with 16 ml binding buffer (4 x 4 ml additions of buffer). The antibody is eluted in one ml fractions with IMMUNOPURE Gentle Elution buffer (Pierce Chemical), and absorbance at 280 nm is determined. Peak fractions are pooled and dialyzed against 50 mM Tris, pH 7.4, 100 mM NaCl, and 10% glycerol. After dialysis, the concentration of the purified antibody is determined using the BCA assay (Pierce Chemical), aliquoted, and frozen.

Electrophoresis and Blotting

[00100] Samples containing protein are mixed in 2 x loading buffer, heated to 95°C for 3-5 min, and loaded on 4-12% NUPAGE Bis-Tris precast gel (Invitrogen). Unless indicated, equal amounts of total protein are loaded into each well. The gel is electrophoresed in 1 x MES or MOPS running buffer (Invitrogen) at 200 V for approximately 45 min on an XCELL II apparatus (Invitrogen) until the RAINBOW marker (GEH) resolves and the dye front approaches the bottom of the gel. The gel is soaked in 1 x transfer buffer (Invitrogen) with 10% methanol for a few minutes; and a PVDF membrane (Millipore, Billerica MA) is soaked in 100% methanol for a few seconds to activate it. The membrane, the gel, and supports are placed on the TRANSBLOT SD transfer apparatus (Biorad, Hercules CA) and a constant current of 350 mA is applied for 90 min.

Conjugation with Antibody and Visualization

[0117] After the proteins are transferred to the membrane, it is blocked in 5% (w/v) non-fat dry milk in 1 x phosphate buffered saline (PBS) with 0.1% Tween 20 detergent (blocking buffer) on a rotary shaker for at least 1 hr at room temperature or at 4°C overnight. After blocking, the buffer is removed, and 10 ml of primary antibody in blocking buffer is added and incubated on the rotary shaker for 1 hr at room temperature or overnight at 4°C. The membrane is washed 3 times for 10 min each with PBS-Tween (PBST), and secondary antibody, conjugated to horseradish peroxidase, is added at a 1:3000 dilution in 10 ml blocking buffer. The membrane and solution are shaken for 30 min at room temperature and washed three times for 10 min with PBST.

[0118] The wash solution is carefully removed, and the membrane is moistened with ECL+ chemiluminescent detection system (GEH) and incubated for approximately 5 min. The

membrane, protein side down, is placed on x-ray film (Eastman Kodak, Rochester NY) and developed for approximately 30 seconds. Antibody:protein complexes are visualized and/or scanned and quantified.

What is claimed is:

1. A method of diagnosing or monitoring steroid responsiveness of a subject comprising:
 - a) detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression is correlated with steroid administration or dosage; and
 - b) applying at least one statistical method to the expression of the diagnostic set to diagnose or monitor steroid responsiveness of the subject.
2. The method of claim 1 wherein the diagnostic set further comprises at least one steroid modulated nucleic acid selected from each of at least two of the clusters of Table 1.
3. The method of claim 1 wherein the diagnostic set further comprises two or more steroid modulated nucleic acids selected from Tables 2 and Table 3.
4. The method of claim 1 wherein detecting the expression further comprises using hybridization or quantitative real-time polymerase chain reaction (RT-PCR).
5. The method of claim 1 wherein the sample further comprises a fluid obtained from the subject by any sampling means.
6. The method of claim 1 wherein the sample is blood containing peripheral blood mononuclear cells (PMBC).
7. The method of claim 1 wherein detecting expression of the diagnostic set of steroid modulated nucleic acids further comprises isolating RNA from the sample.
8. The method of claim 1 wherein the statistical method is K-means clustering or a prediction algorithm.
9. The method of claim 8 wherein K-means clustering produces clusters of genes that are correlated by p-value and their expression in a cell type or pathway.

10. The method of claim 8 wherein the prediction algorithm is selected from a linear algorithm, a logistic regression algorithm, and a voting algorithm and produces a single value or score.
11. The method of claim 1 wherein detecting expression of a diagnostic set further comprises selecting at least two oligonucleotides or a probe set to detect the expression of each nucleic acid of the diagnostic set.
12. A kit comprising the oligonucleotides or probe sets of claim 13.
13. The method of claim 1 wherein diagnosing or monitoring steroid responsiveness further comprises detecting the expression of nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3.
14. A method for predicting rejection or non-rejection in a subject with a transplant comprising:
 - a) detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression of the steroid modulated nucleic acids correlates with transplant rejection or non-rejection; and
 - b) applying at least one statistical method to the expression of the diagnostic set of steroid modulated nucleic acids to predict rejection or non-rejection.
15. The method of claim 14 wherein the diagnostic set of steroid modulated nucleic acids further comprises two or more nucleic acids selected from Tables 1-3.
16. The method of claim 14 wherein the sample is PMBC.
17. The method of claim 14 wherein detecting expression of the diagnostic set of steroid modulated nucleic acids further comprises isolating RNA from the sample.
18. The method of claim 14 wherein detecting expression of the diagnostic set of steroid modulated nucleic acids further comprises using RT-PCR.
19. The method of claim 14 wherein the statistical method is a prediction algorithm that produces single value or score that correlates with rejection or non-rejection.

20. The method of claim 19 wherein the score that correlates with non-rejection is <20 and the score that correlates with rejection is >30 .
21. The method of claim 14 wherein predicting rejection or non-rejection further comprises detecting the expression of a diagnostic set of steroid modulated nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3.
22. A method of diagnosing or monitoring the status of a subject with a transplant comprising:
- a) detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression is correlated with dysfunction or rejection of the transplant; and
 - b) applying at least one statistical method to the expression of the nucleic acids to monitor the status of the transplant.
23. The method of claim 22 wherein the diagnostic set further comprises two or more nucleic acids selected from Tables 1-3.
24. The method of claim 22 wherein the sample is PMBC.
25. The method of claim 22 wherein detecting expression further comprises isolating RNA from the sample.
26. The method of claim 22 wherein detecting expression further comprises using RT-PCR.
27. The method of claim 22 wherein the statistical method is a prediction algorithm that produces single value or score that correlates with the status of a subject with a transplant.
28. The method of claim 22 wherein diagnosing and monitoring the status of a subject with a transplant further comprises detecting the expression of a diagnostic set of steroid modulated nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3.
29. A method for designing and monitoring a treatment plan for a subject with a transplant or an immune disorder comprising:

a) detecting expression of a diagnostic set of at least two steroid modulated nucleic acids in a sample from the subject wherein the expression correlates with the steroid responsiveness of the subject; and

b) using the expression of the diagnostic set of steroid modulated nucleic acids to design and monitor the treatment plan of the subject.

30. The method of claim 29 wherein the diagnostic set of steroid modulated nucleic acids further comprises two or more nucleic acids selected from Tables 1-3.

31. The method of claim 29 wherein the sample is PMBC.

32. The method of claim 29 wherein detecting expression further comprises isolating RNA from the sample.

33. The method of claim 29 wherein detecting expression further comprises using RT-PCR.

34. The method of claim 29 wherein the statistical method is a prediction algorithm.

35. The method of claim 29 wherein diagnosing and monitoring the treatment plan of a subject with a transplant or immune disorder further comprises detecting the expression of a diagnostic set of steroid modulated nucleic acids encoding ADA, CD163, FKBP5, FLT3, FLT3LG, GZMA, IL1R1, IL1R2, ITGAM, NFKB1, PDCD1, THBS1, TNF, TRBC1 and TSC22D3 whose expression correlates with steroid responsiveness of a subject.

36. The method of claim 29 wherein the transplant is selected from bone marrow, heart, kidney, liver, lung, pancreas, pancreatic islets, stem cells, xenotransplants, and artificial implants.

37. The method of claim 29 wherein the immune disorder is selected from cytomegalovirus infection, multiple sclerosis, and systemic lupus erythematosus.

38. A method for using primers and probe sets to detect steroid responsiveness of a subject with a transplant or an immune disorder comprising:

a) designing and generating primers or probe sets for nucleic acids whose expression is modulated by steroid administration or dosage; and

b) using RT-PCR and the primers or probe sets on a sample from the subject to detect steroid responsiveness.

39. The method of claim 38 wherein the nucleic acids whose expression is modulated by steroid administration or dosage are selected from Tables 1-3.