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(54) Title: IN VITRO DIAGNOSIS/PROGNOSIS METHOD AND KIT FOR ASSESSMENT OF CHRONIC ANTIBODY MEDIATED REJECTION IN KIDNEY TRANSPLANTATION

(57) Abstract: This invention refers to the field of human medicine, and specifically to the diagnosis of the rejection state in kidney transplant recipients. More precisely, the present invention concerns a method for the in vitro diagnosis of a kidney graft rejection or non-rejection phenotype, comprising: (a) determining from a kidney grafted subject biological sample an expression profile comprising the 45 genes of Table 1 or subgroups of this list of genes as described in Tables 2, 3 and 4, (b) comparing the obtained expression profile with at least one reference expression profile, and (c) determining the graft rejection or graft non-rejection phenotype from said comparison. The present invention also relates to kits and nucleic acid microarrays for performing said method. The present invention also relates to methods of treatment of kidney transplant recipient.

IN VITRO DIAGNOSIS/PROGNOSIS METHOD AND KIT FOR ASSESSMENT OF CHRONIC ANTIBODY MEDIATED REJECTION IN KIDNEY TRANSPLANTATION

FIELD OF THE INVENTION

This invention refers to the field of human medicine, and specifically to the diagnosis of the rejection state in kidney transplant recipients. More precisely, the present invention concerns a method for the in vitro diagnosis of a kidney graft chronic antibody mediated rejection (then referred as ABMR) or non-rejection phenotype, comprising: (a) determining from a kidney grafted subject biological sample an expression profile comprising the 45 genes of Table 1 or subgroups of this list of genes as described in Tables 2, 3 and 4, (b) comparing the obtained expression profile with at least one reference expression profile, and (c) determining the graft rejection or graft non-rejection phenotype from said comparison. The present invention also relates to kits and nucleic acid microarrays for performing said method. The present invention also relates to methods of treatment of kidney transplant recipients.

STATE OF THE ART

Improvements in immunosuppressive strategies and the management of kidney transplant patients over the last few decades have led to a significant reduction in acute allograft rejection rates and thus an overall improvement in graft survival. However, long-term graft loss remains the bane of kidney transplantation. The causes of late graft loss are varied, with a major implication of toxicity caused by calcineurin inhibitors (CNIs) as well as multiple factors leading to interstitial fibrosis and tubular atrophy. More recently, much attention has been paid to the "humoral" theory of chronic allograft rejection. Evidence for the involvement of a humoral arm of the immune response to allografts has come from studies analyzing the impact of anti-HLA antibodies on graft outcome and evidence of complement cascade activation within kidney grafts diagnosed by intragraft deposit of the complement split product C4d. These data were recently reinforced when the definition of *chronic active antibody*

mediated rejection (chronic AMBR) was introduced into the Banff classification of kidney graft injury. chronic AMBR is defined by the diagnostic association of specific histological lesions associated with diffuse C4d deposit in peritubular capillaries and circulating donor-specific anti-HLA antibodies. The diagnosis of "Suspicious for chronic AMBR" is given in the case of morphologic evidence of tissue injury where C4d is positive but donor specific antibodies (DSA) are absent, or if DSA are present but C4d is negative. Thus, the diagnosis of chronic AMBR requires both an invasive biopsy and a non-invasive blood test. The biopsy serves to identify the presence of specific lesions and complement deposit by specific histology and immunohistology techniques. The blood test serves to detect the presence of circulating donor-specific antibodies using high-definition *in vitro* methods. As the incidence of chronic AMBR appears to increase throughout the post transplant course, the majority of cases are detected upon biopsy for graft deterioration i.e. biopsy for cause.

It would thus be very valuable to have a non-invasive method allowing diagnosing chronic antibody mediated rejection at the earlier steps of the rejection process, which would permit to adapt the immunosuppressive treatment and might in some cases prevent the deterioration of graft function. Such a method should be sensitive enough to detect chronic rejection at its earlier steps. It should also preferably be non invasive, which would permit to perform control tests on a regular basis, without affecting the grafted subject's quality of life or endangering the survival of the graft.

Altogether, there is thus a need for a non invasive, simple, easy to perform and reliable method for diagnosing chronic antibody mediated rejection in a grafted subject based on a biological sample of said subject.

DESCRIPTION OF THE INVENTION

Interestingly, the inventors found that chronic antibody mediated rejection (chronic AMBR) of kidney transplanted patients to their kidney graft could be diagnosed or prognosed based on the simple analysis of the expression level in blood cells of a set of 45 genes, and even sub-sets of 29 genes, 15 genes or 14 genes, or equivalents thereof, and comparison with the expression level in blood cells of the same 45, 29, 15 or 14 genes in reference blood samples of chronic ABMR and non-chronic ABMR patients.

The invention thus relates to a method for the in vitro diagnosis of a kidney graft rejection or non-rejection phenotype, comprising:

(a) determining from a kidney grafted subject biological sample an expression profile comprising or consisting of the 45 genes of following Table 1 or of the subsets of 29, 15 or 14 genes following Tables 2, 3 or 4, or equivalents thereof,

(b) comparing the obtained expression profile with at least one reference expression profile, and

(c) determining the rejection or non-rejection phenotype from said comparison.

The invention also relates to a method for designing a kidney transplant recipient immunosuppressive treatment, said method comprising:

(a) Determining from a biological sample of said kidney transplant recipient an expression profile comprising or consisting of the 45 genes of following Table 1, or of the subsets of 29, 15 or 14 genes following Tables 2, 3 or 4, or equivalents thereof,

(b) Comparing the obtained expression profile with at least one reference expression profile,

(c) Determining the graft rejection or graft non-rejection phenotype of said kidney transplant recipient from said comparison, and

(d) Designing a dose of immunosuppressive drug treatment according to the said identified graft rejection or graft non-rejection phenotype.

The invention also relates to a method for adapting the immunosuppressive treatment of a kidney transplant recipient, said method comprising:

(a) Determining from a biological sample of said kidney transplant recipient an expression profile comprising or consisting of the 45 genes of following Table 1, or of the subsets of 29, 15 or 14 genes following Tables 2, 3 or 4, or equivalents thereof,

(b) Comparing the obtained expression profile with at least one reference expression profile,

(c) Determining the graft rejection or graft non-rejection phenotype kidney transplant recipient from said comparison, and

(d) Adapting accordingly the immunosuppressive treatment.

The invention is also drawn to a method for treating of a kidney transplant recipient, comprising:

(a) determining from a biological sample of said kidney transplant recipient the presence of a graft rejection or graft non-rejection phenotype using a method according to the invention, and

(b) adapting the immunosuppressive treatment in function of the result of step (a).

Said adaptation of the immunosuppressive treatment may consist in:

- a modification of said immunosuppressive treatment if the subject has been diagnosed as graft rejection, or
- a maintenance of said immunosuppressive treatment if the subject has been diagnosed as graft non rejection.

The present invention presents a major interest. It permits to diagnose or prognose (i.e. to identify), among patients under immunosuppressive treatment, those who are in the process of rejecting their graft and who could thus benefit from an adapted immunosuppressive treatment dedicated to stop or at least slow down the rejection process. Due to the non reversible aspects of the damages caused by chronic rejection and the current late diagnosis of such phenotype, this achievement is really crucial and would allow a better management of the patients.

According to the invention, a "kidney transplanted subject" or a "kidney transplant recipient" is a subject that was grafted with a non syngeneic, including allogenic or even xenogenic, kidney. Said kidney transplanted subject may further have been grafted with another organ of the same donor providing the kidney.

According to the present invention, a "graft rejection phenotype" is defined as a state of chronic antibody mediated rejection of a subject to his graft. A "state of rejection" means that this subject (referred to as a "graft rejecting subject") is rejecting his graft according to the Banff classification. Such phenotype can correspond either to a strict chronic antibody mediated rejection definition or a "suspicious for ABMR"

definition. In contrast, a "graft non-rejection phenotype" refers to the absence in said subject of a state of rejection, meaning that said subject (referred to as a "graft non-rejecting subject") would, at the time of the diagnosis, not reject his/her graft. While the population of graft rejecting subjects only includes subjects in a state of rejection to their graft, the population of graft non-rejecting subjects thus includes all other subjects and is composed of a variety of different states such as patients with signs of toxicity of Calcineurin inhibitors, patients with signs of interstitial fibrosis and/or tubular atrophy without C4d deposit nor anti-HLA antibodies but also stable patients with or without anti-HLA antibodies, who cannot at this time be considered as rejecting but who may later develop a graft rejection phenotype. Indeed, it must be understood that the mechanisms of rejection are complex and still not elucidated, and the cellular and molecular processes of rejection induction may require a prolonged laps of time. Thus, while the population of graft rejecting subjects only includes subjects who have already reached a state of ABMR or suspicion of ABMR to their graft, the population of graft non-rejecting subjects is heterogeneous and includes all other subjects, i.e. both subjects with renal failure for other causes than ABMR and subjects with stable graft function.

A "biological sample" may be any sample that may be taken from a grafted subject, such as a serum sample, a plasma sample, a urine sample, a blood sample, a lymph sample, or a biopsy. Preferred biological samples for the determination of an expression profile include a blood sample, a lymph sample, or a biopsy. Preferably, the biological sample is a blood sample, more preferably a peripheral blood sample comprising peripheral blood mononuclear cells (PBMC) or whole blood. Indeed, such blood samples may be obtained by a completely harmless blood collection from the grafted patient and thus allows for a non-invasive diagnosis of a graft rejection or non-rejection phenotype.

By "expression profile" is meant the expression levels of a group of genes comprising the 45 genes of Table 1 or a subgroup thereof. In a most preferred embodiment, the expression profile consists of the 45 genes of Table 1, of the 29 genes of Table 2, of the 15 genes of Table 3 or of the 14 genes of Table 4 or Equivalent

Expression Profiles thereof, since these expression profiles have been demonstrated to be particularly relevant for assessing kidney graft rejection/non-rejection.

By "Equivalent Expression Profile" is meant expression profiles of tables 1, 2, 3 or 4 wherein the addition, deletion or substitution of some of the genes does not change significantly the reliability of the test and is considered as an "acceptable expression profile". As an example, the addition of other genes already described as significant in chronic AMBR diagnostic, such as TRIB 1 (WO 2007/138011) or PSMB10 (EP08300084) should be considered as an equivalent. As another example, the addition or substitution of some of the genes of the sets described in the present invention by other genes belonging to the same metabolic pathway should also be considered as an equivalent expression profile.

By "Acceptable Expression Profile" is meant an expression profile which is able to correctly classify at least 60% of the analyzed samples, preferably 65%, and more preferably 70%, has a sensitivity of at least 60% preferably 65%, and more preferably 70% and has a negative predictive value of at least 70%, preferably 80%, and more preferably 90%. The sensitivity value is defined as the ratio of the number of patients actually clinically rejecting their graft due to chronic ABMR and classified as rejecting using the test according to the invention amongst all patients analysed displaying clinical signs of ABMR. The negative predictive value is defined as the ratio of the number of patients clinically defined as non rejecting ones and classified as non-rejecting using the test according to the invention amongst the total number of patients classified as non-rejecting using the test according to the invention.

By "Best Expression Profile" is meant an expression profile which is able to correctly classify at least 70% of the analyzed samples, has a sensitivity of at least 70%, and has a negative predictive value of at least 90%.

Although the list of 45 genes of Table 1 has been determined as the Best Expression Profile to assess graft rejection/non-rejection, an Equivalent Expression Profile such as defined above, still permits to assess graft rejection, with an acceptable reliability. In particular embodiments, sublists of the 45 genes such as the ones described in Tables 2, 3 and 4 still permits to assess graft rejection with a good reliability and should be considered as Acceptable Expression Profiles.

The 45 genes Expression Profile that were determined by the inventors to display to be able to discriminate between kidney graft rejecting subjects as defined above and kidney graft non-rejecting subjects are listed in the following Table 1.

Table 1

N°	Symbol	Name	AccessionNb
1	DDX6	CDNA FLJ11653 fis, clone HEMBA1004538	NM_004397.4
2	HBB	hemoglobin, beta	NM_000518.4
3	HINT3	histidine triad nucleotide binding protein 3	NM_138571.4
4	ITGA6	Integrin, alpha 6	NP_000201.2
5	NKTR	natural killer-tumor recognition sequence	NM_005385.3
6	PDZK1IP 1	PDZK1 interacting protein 1	NM_005764.3
7	YEATS2	YEATS domain containing 2	NM_018023.3
8	ZBTB16	zinc finger and BTB domain containing 16	NM_001018011.1 NM_006006.4
9	BRAF	v-raf murine sarcoma viral oncogene homolog B1	NM_004333.4
10	FOXP1	forkhead box P1	NM_001012505.1 NM_032682.4
11	CST3	Transcribed locus	NM_000099.2
12	ADNP	Activity-dependent neuroprotector	NM_015339.2 NM_181442.1
13	ARHGEF 7	Rho guanine nucleotide exchange factor (GEF) 7	NM_001113511.1 NM_001113512.1 NM_001113513.1 NM_003899.3 NM_145735.2
14	CLASP1	Cytoplasmic linker associated protein 1	NM_001142273.1 NM_001142274.1 NM_015282.2
15	EDEM1	ER degradation enhancer, mannosidase alpha-like 1	NM_014674.2
16	KIAA0256	KIAA0256 gene product	NM_014701.2
17	ANKRD3 6B	KIAA1641	NM_025190.2
18	SMG1	hypothetical protein LOC440345 /similar to PI-3-kinase-related kinase SMG-1	NM_015092.4
19	MRCL3	Hypothetical protein LOC727918	NM_006471.2

20	MALAT1/ SLC25A4 5	Metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)	NM_001077241.1 NM_182556.2
21	MDM4	Mdm4, transformed 3T3 cell double minute 4, p53 binding protein (mouse)	NM_002393.3
22	MSI2	musashi homolog 2 (Drosophila)	NM_138962.2 NM_170721.1
23	OXNAD1	Oxidoreductase NAD-binding domain containing 1	NM_138381.2
24	SFRS11	Splicing factor, arginine/serine-rich 11	NM_004768.2
25	STX16	syntaxin 16	NM_001001433 NM_003763
26	TOB1	transducer of ERBB2, 1	NM_005749.2
27	ZBTB11	Zinc finger and BTB domain containing 11	NM_014415.3
28	ZBTB25	Zinc finger and BTB domain containing 25	NM_006977.2
29	LATS2	LATS, large tumor suppressor, homolog 2 (Drosophila)	NM_014572.2
30	PRKCD	protein kinase C, delta	NM_006254.3 NM_212539.1
31	CSNK1G 3	casein kinase 1, gamma 3	NM_001031812.2 NM_001044722.1 NM_001044723.1 NM_004384.3
32	MAP3K2	mitogen-activated protein kinase kinase kinase 2	NM_006609.3
33	ZBTB44	zinc finger and BTB domain containing 44	NM_014155.4
34	COCH	coagulation factor C homolog, cochlin (Limulus polyphemus)	NM_004086
35	ALPK1	alpha-kinase 1	NM_001102406.1 NM_025144.3
36	FAM60A : LOC6503 69 : LOC7281 15	family with sequence similarity 60, member A /// similar to teratocarcinoma expressed, serine rich /// similar to Protein FAM60A (Tera protein)	NM_021238
37	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	NM_005238
38	TLR2	toll-like receptor 2	NM_003264.3
39	NCF1 : NCF1B : NCF1C	neutrophil cytosolic factor 1, (chronic granulomatous disease, autosomal 1) /// neutrophil cytosolic factor 1B pseudogene	NM_000265.4
40	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	NM_022898.1 NM_138576.2
41	LY9	lymphocyte antigen 9	NM_001033667.1 NM_002348.2

42	RRAS	related RAS viral (r-ras) oncogene homolog	NM_006270.3
43	PTPRC	protein tyrosine phosphatase, receptor type, C	NM_002838.3 NM_080921.2 NM_080922.2 NM_080923.2
44	GABPB2	GA binding protein transcription factor, beta subunit 2	NM_144618.2
45	TCF7	transcription factor 7 (T-cell specific, HMG-box)	NM_003202 NM_201632 NM_201633 NM_201634 NM_213648

The subsets of 29, 15 and 14 genes that were determined by the inventors to be able to discriminate between kidney graft rejecting subjects as defined above and kidney graft non-rejecting subjects are listed respectively in the following Table 2, 3 and 4.

Table 2

N°	Symbol	Name	AccessionNb
1	DDX6	CDNA FLJ11653 fis, clone HEMBA1004538	NM_004397.4
2	HBB	hemoglobin, beta	NM_000518.4
3	ITGA6	Integrin, alpha 6	NP_000201.2
4	ZBTB16	zinc finger and BTB domain containing 16	NM_001018011.1 NM_006006.4
5	BRAF	v-raf murine sarcoma viral oncogene homolog B1	NM_004333.4
6	FOXP1	forkhead box P1	NM_001012505.1 NM_032682.4
7	ADNP	Activity-dependent neuroprotector	NM_015339.2 NM_181442.1
8	ARHGEF 7	Rho guanine nucleotide exchange factor (GEF) 7	NM_001113511.1 NM_001113512.1 NM_001113513.1 NM_003899.3 NM_145735.2
9	CLASP1	Cytoplasmic linker associated protein 1	NM_001142273.1 NM_001142274.1 NM_015282.2
10	SMG1	hypothetical protein LOC440345 /similar to PI-3-kinase-related kinase SMG-1	NM_015092.4
11	MDM4	Mdm4, transformed 3T3 cell double minute 4, p53 binding protein (mouse)	NM_002393.3

12	MSI2	musashi homolog 2 (Drosophila)	NM_138962.2 NM_170721.1
13	SFRS11	Splicing factor, arginine/serine-rich 11	NM_004768.2
14	STX16	syntaxin 16	NM_001001433 NM_003763
15	TOB1	transducer of ERBB2, 1	NM_005749.2
16	ZBTB11	Zinc finger and BTB domain containing 11	NM_014415.3
17	LATS2	LATS, large tumor suppressor, homolog 2 (Drosophila)	NM_014572.2
18	PRKCD	protein kinase C, delta	NM_006254.3 NM_212539.1
19	CSNK1G3	casein kinase 1, gamma 3	NM_001031812.2 NM_001044722.1 NM_001044723.1 NM_004384.3
20	MAP3K2	mitogen-activated protein kinase kinase kinase 2	NM_006609.3
21	ZBTB44	zinc finger and BTB domain containing 44	NM_014155.4
22	COCH	coagulation factor C homolog, cochlin (Limulus polyphemus)	NM_004086
23	FAM60A : LOC650369 : LOC728115	family with sequence similarity 60, member A /// similar to teratocarcinoma expressed, serine rich /// similar to Protein FAM60A (Tera protein)	NM_021238
24	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	NM_005238
25	TLR2	toll-like receptor 2	NM_003264.3
26	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	NM_022898.1 NM_138576.2
27	RRAS	related RAS viral (r-ras) oncogene homolog	NM_006270.3
28	PTPRC	protein tyrosine phosphatase, receptor type, C	NM_002838.3 NM_080921.2 NM_080922.2 NM_080923.2
29	TCF7	transcription factor 7 (T-cell specific, HMG-box)	NM_003202 NM_201632 NM_201633 NM_201634 NM_213648

Table 3

N°	Symbol	Name	AccessionNb
1	HBB	hemoglobin, beta	NM_000518.4
2	ITGA6	Integrin, alpha 6	NP_000201.2
3	BRAF	v-raf murine sarcoma viral oncogene homolog B1	NM_004333.4
4	ARHGEF 7	Rho guanine nucleotide exchange factor (GEF) 7	NM_001113511.1 NM_001113512.1 NM_001113513.1 NM_003899.3 NM_145735.2
5	MDM4	Mdm4, transformed 3T3 cell double minute 4, p53 binding protein (mouse)	NM_002393.3
6	TOB1	transducer of ERBB2, 1	NM_005749.2
7	PRKCD	protein kinase C, delta	NM_006254.3 NM_212539.1
8	MAP3K2	mitogen-activated protein kinase kinase kinase 2	NM_006609.3
9	ZBTB44	zinc finger and BTB domain containing 44	NM_014155.4
10	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	NM_005238
11	TLR2	toll-like receptor 2	NM_003264.3
12	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	NM_022898.1 NM_138576.2
13	RRAS	related RAS viral (r-ras) oncogene homolog	NM_006270.3
14	PTPRC	protein tyrosine phosphatase, receptor type, C	NM_002838.3 NM_080921.2 NM_080922.2 NM_080923.2
15	TCF7	transcription factor 7 (T-cell specific, HMG-box)	NM_003202 NM_201632 NM_201633 NM_201634 NM_213648

Table 4

N°	Symbol	Name	AccessionNb
1	DDX6	CDNA FLJ11653 fis, clone HEMBA1004538	NM_004397.4
2	ZBTB16	zinc finger and BTB domain containing 16	NM_001018011.1 NM_006006.4

3	FOXP1	forkhead box P1	NM_001012505.1 NM_032682.4
4	ADNP	Activity-dependent neuroprotector	NM_015339.2 NM_181442.1
5	CLASP1	Cytoplasmic linker associated protein 1	NM_001142273.1 NM_001142274.1 NM_015282.2
6	SMG1	hypothetical protein LOC440345 /similar to PI-3-kinase-related kinase SMG-1	NM_015092.4
7	MSI2	musashi homolog 2 (Drosophila)	NM_138962.2 NM_170721.1
8	SFRS11	Splicing factor, arginine/serine-rich 11	NM_004768.2
9	STX16	syntaxin 16	NM_001001433 NM_003763
10	ZBTB11	Zinc finger and BTB domain containing 11	NM_014415.3
11	LATS2	LATS, large tumor suppressor, homolog 2 (Drosophila)	NM_014572.2
12	CSNK1G3	casein kinase 1, gamma 3	NM_001031812.2 NM_001044722.1 NM_001044723.1 NM_004384.3
13	COCH	coagulation factor C homolog, cochlin (Limulus polyphemus)	NM_004086
14	FAM60A : LOC650369 : LOC728115	family with sequence similarity 60, member A /// similar to teratocarcinoma expressed, serine rich /// similar to Protein FAM60A (Tera protein)	NM_021238

Based on gene networks represented in Figures 1 and 2, Equivalent Expression Profiles of the profiles obtained using the gene sets of Tables 3 or 4 respectively may be easily obtained by a skilled artisan:

- by addition to the gene sets of Table 3 or 4 of one or more genes depicted in Figure 1 or 2 respectively, or
- by substitution of one or more genes of the genes sets of Table 3 or 4 by one or more genes depicted in Figure 1 or 2 respectively.

In the same manner, based on gene networks represented in Figures 1 and 2, Equivalent Expression Profiles of the profiles obtained using the gene sets of Tables 1 or 2 may be easily obtained by a skilled artisan:

- by addition to the gene sets of Table 1 or 2 of one or more genes depicted in Figures 1 and 2, or

- by substitution of one or more genes of the genes sets of Table 1 or 2 by one or more genes of the same metabolic pathway as depicted in Figures 1 and 2.

The determination of the presence of a graft rejection or graft non-rejection phenotype is carried out thanks to the comparison of the obtained expression profile with at least one reference expression profile in step (b).

A "reference expression profile" is a predetermined expression profile, obtained from a biological sample from a subject with a known particular graft state. In particular embodiments, the reference expression profile used for comparison with the test sample in step (b) may have been obtained from a biological sample from a graft rejecting subject ("rejecting reference expression profile"), and/or from a biological sample from a graft non-rejecting subject ("non-rejecting reference expression profile").

Preferably, at least one reference expression profile is a rejecting reference expression profile. Alternatively, at least one reference expression profile may be a non-rejecting reference expression profile. More preferably, the determination of the presence or absence of a graft rejection phenotype is carried out by comparison with at least one rejecting and at least one non-rejecting reference expression profiles. The diagnosis (or prognostic) may thus be performed using one rejecting reference expression profile and one non-rejecting reference expression profile. Advantageously, to get a stronger diagnosis, said diagnosis is carried out using several rejecting reference expression profiles and several non-rejecting reference expression profiles.

The comparison of a tested subject expression profile with said reference expression profiles can be done using statistical models or machine learning methods which aim is to predict a clinical response (eg: 0 if Not-rejecting, 1 if rejecting) based on a combination of the explanatory variables (the genes). Statistical models such as logistic regression and fisher linear discriminant analysis are particularly relevant to predict outcome. Other discriminating algorithms include kNN (k nearest neighbour), decision trees, SVM (support vector machine), NN (neural networks) and forest. The PLS regression, MIPP, sparse linear discrimination and PAM (predictive analysis of microarrays) are particularly relevant to give prediction in the case of pangenomic analyses with small reference samples. To ensure that the predictor is robust, cross validation methods such as leave-one-out should be applied to the models.

The expression profile may be determined by any technology known by a man skilled in the art. In particular, each gene expression level may be measured at the genomic and/or nucleic and/or proteic level.

In a preferred embodiment, the expression profile is determined by measuring the amount of nucleic acid transcripts of each gene. In another embodiment, the expression profile is determined by measuring the amount of each gene corresponding protein.

The amount of nucleic acid transcripts can be measured by any technology known by a man skilled in the art. In particular, the measure may be carried out directly on an extracted messenger RNA (mRNA) sample, or on retrotranscribed complementary DNA (cDNA) prepared from extracted mRNA by technologies well-know in the art. From the mRNA or cDNA sample, the amount of nucleic acid transcripts may be measured using any technology known by a man skilled in the art, including nucleic microarrays, quantitative PCR, and hybridization with a labelled probe.

In a preferred embodiment, the expression profile is determined using quantitative PCR. Quantitative, or real-time, PCR is a well known and easily available technology for those skilled in the art and does not need a precise description.

In a particular embodiment, which should not be considered as limiting the scope of the invention, the determination of the expression profile using quantitative PCR may be performed as follows. Briefly, the real-time PCR reactions are carried out using the TaqMan Universal PCR Master Mix (Applied Biosystems). 6 µl cDNA is added to a 9 µl PCR mixture containing 7.5 µl TaqMan Universal PCR Master Mix, 0.75 µl of a 20X mixture of probe and primers and 0.75µl water. The reaction consisted of one initiating step of 2 min at 50 deg. C, followed by 10 min at 95 deg. C, and 40 cycles of amplification including 15 sec at 95 deg. C and 1 min at 60 deg. C. The reaction and data acquisition can be performed using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems). The number of template transcript molecules in a sample is determined by recording the amplification cycle in the exponential phase (cycle threshold or C_T), at which time the fluorescence signal can be detected above

background fluorescence. Thus, the starting number of template transcript molecules is inversely related to C_T.

In another preferred embodiment, the expression profile is determined by the use of a nucleic microarray.

According to the invention, a "nucleic microarray" consists of different nucleic acid probes that are attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. A microchip may be constituted of polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, or nitrocellulose. Probes can be nucleic acids such as cDNAs ("cDNA microarray") or oligonucleotides ("oligonucleotide microarray"), and the oligonucleotides may be about 25 to about 60 base pairs or less in length.

To determine the expression profile of a target nucleic sample, said sample is labelled, contacted with the microarray in hybridization conditions, leading to the formation of complexes between target nucleic acids that are complementary to probe sequences attached to the microarray surface. The presence of labelled hybridized complexes is then detected. Many variants of the microarray hybridization technology are available to the man skilled in the art.

In a preferred embodiment, the nucleic acid microarray is an oligonucleotide microarray comprising or consisting of 45 oligonucleotides specific for the 45 genes of Table 1, or comprising or consisting of 29 oligonucleotides specific for the 29 genes of Table 2, or comprising or consisting of 15 oligonucleotides specific for the 15 genes of Table 3, or comprising or consisting of 14 oligonucleotides specific for the 14 genes of Table 4. Preferably, the oligonucleotides are about 50 bases in length. It is acknowledged that the nucleic acid microarray, or oligonucleotide microarray of the invention encompass the microarrays specific for an Equivalent Expression Profile as defined above.

Suitable oligonucleotides specific for any gene of Table 1, 2, 3 or 4 may be designed, based on the genomic sequence of each gene (see Genbank accession numbers), using any method of microarray oligonucleotide design known in the art. In particular, any available software developed for the design of microarray oligonucleotides may be used, such as, for instance, the OligoArray software (available at http://berry.engin.umich.edu/oligoarray/), the GoArrays software (available at

http://www.isima.fr/bioinfo/goarrays/), the Array Designer software (available at http://www.premierbiosoft.com/dnamicroarray/index.html), the Primer3 software (available at http://frodo.wi.mit.edu/primer3/primer3_code.html), or the Promide software (available at http://oligos.molgen.mpg.de/).

In another preferred embodiment, the expression profile is determined by the use of proteic microarrays. For expression profiling experiments, antibodies, aptamers, or affibodies microarrays are mainly used, most of the time antibodies microarrays (Hall et al, 2007). The antibodies, aptamers, or affibodies are attached to various supports using various attachment methods, using a contact or non-contact spotter (Hall et al, 2007). Examples of suitable supports include glass and silicon microscope slides, nitrocellulose, microwells (for instance made of a silicon elastomer) (Hall et al, 2007). For glass and silicon microscope slides, a coating is generally added. Examples of coatings for random attachment (i.e. resulting in a random orientation of attached proteins to the support) include aldehyde- and epoxy-derivatized coatings for random attachment through amines, and nitrocellulose, gel pads or poly-L-lysine coatings (Hall et al, 2007). Examples of coatings for non random attachment (i.e. resulting in a uniform orientation of attached proteins to the support) include nickel coating fro use with His6-tag proteins, and streptavidin coating for use with biotinylated proteins (Hall et al, 2007). For detection, two main technologies are used: 1) direct labelling, single capture assays and 2) dual-antibody sandwich immunoassays (Kingsmore, 2006, see notably Figure 1). In direct labelling, single capture assays, proteins contains in one or more samples are labelled with distinct labels (generally fluorescent or radioisotope labels), hybridized to the microarray, and labelled hybridized proteins are directly detected (Kingsmore, 2006, see notably Figure 1a). In dual-antibody sandwich immunoassays, the sample is hybridized to the microarray, and a secondary tagged antibody is added. A third labelled (generally fluorescent or radioisotope label) antibody specific for the tag of the secondary antibody is then used for detection (Kingsmore, 2006, see notably Figure 1b). Further details concerning antibodies microarrays may be found in Haab, 2005 and Eckel-Passow et al, 2005. Examples of commercial antibody microarrays include those commercialized by Clontech Laboratories, Invitrogen, Eurogentec, Kinexus etc…

In a particular embodiment of a method according to the invention, said method may further comprise determining from a non invasive biological sample of the subject at least one additional parameter useful for the diagnosis. Such "parameters useful for the diagnosis" are parameters that cannot be used alone for a diagnosis but that have been described as displaying significantly different values between grafted subjects in chronic antibody mediated rejection and non-rejecting subjects and may thus also be used to refine and/or confirm the diagnosis according to the above described method according to the invention. They may notably be selected from:

- standard biological and histological parameters specific for said subject grafted organ type,
- phenotypic analyses of peripheral blood cells, and
- qualitative and/or quantitative analysis of peripheral blood cells immune repertoire.

According to the invention, "standard biological parameters specific for said subject grafted organ type" means biological parameters that are usually used by clinicians to monitor the stability of grafted subjects status and to detect chronic antibody mediated rejection. These standard biological parameters specific for said subject grafted organ usually comprise serum or plasma concentrations of particular proteins as well as the presence of anti-HLA antibodies, which vary depending on the grafted organ type. However, these standard biological parameters specific for said subject grafted organ type are, for each organ type, well known of those skilled in the art.

For instance, standard biological parameters specific for kidney include serum or plasma urea and creatinine concentrations. In a healthy subject, the serum creatinine concentration is usually comprised between 40 to 80 µmol/L for a woman and 60 to 100 µmol/L for a man, and the serum urea concentration between 4 to 7 mmol/L. A serum creatinine concentration superior to 80 µmol/L for a woman and to 100 µmol/L for a man, or a serum urea concentration superior to 7 mmol/L may confirm a chronic rejection status.

These standard biological parameters have the advantage of being easily measurable from a blood sample, but are not sufficient to establish a precise chronic

antibody mediated rejection or non-rejection diagnosis without the information from the analysis of the biopsy, and are also not enough sensitive to allow an early chronic rejection diagnosis. However, when combined with the determination of an expression profile according to the present invention, the resulting method according to the invention makes it possible to detect patients at risk of chronic antibody mediated rejection without requiring a biopsy, as well as patients with no signs of chronic antibody mediated rejection.

The phenotypic analyses of peripheral blood mononuclear cells (PBMC) may comprise various types of phenotypic analysis. In particular they may comprise:

- measuring the percentage of $CD4^+$ $CD25^+$ T cells in peripheral blood lymphocytes, which may be performed by any technology known in the art, in particular by flow cytometry using labelled antibodies specific for the CD4 and CD25 molecules. Preferably, the percentage of $CD4^+$ $CD25^+$ T cells in peripheral blood lymphocytes of a subject undergoing chronic rejection is significantly lower ($p < 0.05$) from that of a healthy volunteer.
- determining the cytokine expression profile of T cells, which may be performed using any technology known in the art, including quantitative PCR and flow cytometry analysis. Preferably, the oligoclonal Vβ families of a grafted subject undergoing chronic rejection express increased levels compared to a healthy volunteer of TH1 or TH2 effector molecules, including interleukin 2 (IL-2), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 13 (IL-13), transforming growth factor beta (TGF-β), interferon gamma (IFN-γ) and perforin, whereas oligoclonal Vβ families of a grafted subject with stable graft function do not express increased levels of those effector molecules compared to a healthy volunteer.

The analysis of peripheral blood cells immune repertoire consists advantageously in the qualitative and quantitative analysis of the T cell repertoire (2), such as the T cell repertoire oligoclonality and the level of TCR transcripts or genes.

The T cell repertoire oligoclonality may be determined by any technology enabling to quantify the alteration of a subject T cell repertoire diversity compared to a control repertoire. Usually, said alteration of a subject T cell repertoire diversity

compared to a control repertoire is determined by quantifying the alteration of T cell receptors (TCR) complementary determining region 3 (CDR3) size distributions. In a healthy subject, who can be considered as a control repertoire, such a TCR CDR3 size distribution displays a Gaussian form, which may be altered in the presence of clonal expansions due to immune response, or when the T cell repertoire diversity is limited and reaches oligoclonality.

The level of TCR expression at the genomic, transcriptomic or proteic level is preferably determined independently for each Vβ family by any technology known in the art. For instance, the level of TCR transcripts of a particular Vβ family may be determined by calculating the ratio between these Vβ transcripts and the transcripts of a control housekeeping gene, such as the HPRT gene. Preferably, in a graft rejecting subject, a significant percentage of Vβ families display an increase in their transcript numbers compared to patients with stable kidney graft function or to healthy individuals.

An example of methods to analyze T cell repertoire oligoclonality and/or the level of TCR transcripts, as well as scientific background relative to T cell repertoire, are clearly and extensively described in WO 02/084567 (24), which is herein incorporated by reference.

Such additional parameters may be used to confirm the diagnosis obtained using the expression profile comprising or consisting of the 45 genes from Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4, or Equivalent Expression Profiles.

The invention further concerns a kit for the in vitro diagnosis of a graft rejection or graft non-rejection phenotype, comprising at least one reagent for the determination of an expression profile comprising, or consisting of, the 45 genes from Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4, or Equivalent Expression Profiles thereof. By "a reagent for the determination of an expression profile" is meant a reagent which specifically allows for the determination of said expression profile, i.e. a reagent specifically intended for the specific determination of the expression level of the genes comprised in the expression profile, either on the

transcription (RNA) of the translation (proteic) levels. This definition excludes generic reagents useful for the determination of the expression level of any gene, such as taq polymerase or an amplification buffer, although such reagents may also be included in a kit according to the invention.

Such a kit for the in vitro diagnosis of a graft rejection or graft non-rejection phenotype may further comprise instructions for determination of the presence or absence of a graft rejection phenotype.

Such a kit for the in vitro diagnosis of a graft rejection phenotype may also further comprise at least one reagent for the determining of at least one additional parameter useful for the diagnosis such as standard biological parameters specific for said subject grafted organ type (notably the presence of anti-HLA antibodies), phenotypic analyses of peripheral blood cells, and quantitative and/or qualitative analysis of peripheral blood cells immune repertoire (such as the T cell repertoire oligoclonality and the level of TCR transcripts).

In any kit for the in vitro diagnosis of a graft rejection phenotype according to the invention, the reagent(s) for the determination of an expression profile comprising, or consisting of, the 45 genes from Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4 or Equivalent Expression Profiles thereof, preferably include specific amplification primers and/or probes for the specific quantitative amplification of transcripts of genes of Table 1, and/or a nucleic microarray for the detection of genes of Table 1. The determination of the expression profile may thus be performed using quantitative PCR and/or a nucleic microarray, preferably an oligonucleotide microarray.

In addition, the instructions for the determination of the presence or absence of a graft rejection phenotype preferably include at least one reference expression profile, or at least one reference sample for obtaining a reference expression profile. In a preferred embodiment, at least one reference expression profile is a graft rejection expression profile. Alternatively, at least one reference expression profile may be a graft non-rejection expression profile. More preferably, the determination of the level of graft rejection is carried out by comparison with both graft rejection and graft non-rejection expression profiles as described above.

The invention is also directed to a nucleic acid microarray comprising or consisting of nucleic acids specific for the 45 genes from Table 1, or subsets of these 45 genes such as those described in Tables 2, 3 and 4 or Equivalent Expression Profiles thereof. Said nucleic acid microarray may comprise additional nucleic acids specific for genes other than the 45 genes from Table 1, but preferably consists of a maximum of 500, 400, 300, 200 preferably 100, 90, 80, 70 more preferably 60, 50, or even 45 distinct nucleic acids, 45 of which are specific for the 45 genes of Table 1. When subsets of genes of Table 1 are used, the nucleic acid microarray may include even less distinct nucleic acids. Advantageously, said microarray consists of nucleic acids specific for the 45 genes of Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4. In a preferred embodiment, said nucleic acid microarray is an oligonucleotide microarray comprising or consisting of oligonucleotides specific for the 45 genes from Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4, or for Equivalent Expression Profiles thereof.

Having generally described this invention, a further understanding of characteristics and advantages of the invention can be obtained by reference to certain specific examples and figures which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

DESCRIPTION OF THE FIGURES

Figure 1. Scheme of a first network of interacting genes.

Figure 2. Scheme of a second network of interacting genes.

In both Figures 1 and 2, symbols represent different types of molecules;

- upward triangles: phosphatase,
- downward triangles: kinase,
- horizontal ellipses: transcription regulator,
- vertical ellipses: transmembrane receptor,
- rhombuses: enzyme,
- horizontal rectangles: ligand dependent nuclear receptor,
- vertical rectangles: g-protein coupled receptor,

- simple circles: other types of molecules,
- double circles: complex group,

Shaded shapes represent the molecules that are part of the 45 list.

The types of line represent the type of interaction:

- plain arrows: means the first molecule directly acts on the second one (such as but not limited to activation)
- dotted arrows: means the first molecule indirectly acts on the second one
- A line ending with a short segment indicates inhibition
- A simple line represents interaction (such a but not limited to protein-protein interaction)

EXAMPLES

EXAMPLE 1. Patients

Peripheral blood samples from 19 patients with ABMR and 124 relevant controls (transplant patients with various other clinical statuses or histological diagnoses – see Table 5) were identified and analyzed by microarrays (HG-U133 Plus2) on an Affymetrix platform. The group of patients with ABMR was composed both of a group at 1yr post transplant in patients with no renal dysfunction (protocol biopsies), and another group at a median of 5 years post transplant with renal failure (biopsies for cause).

Table 5

Time Post-Transplant	Patient Group	n
Chronic antibody-mediated rejection		n=19
1-yr	Chronic antibody-mediated rejection	6
median 5 years	Chronic antibody-mediated rejection	13
Control group		n=124
NA	Non transplant renal failure	14
median 5 years	Stable graft function without circulating anti-HLA	33
median 5 years	Stable graft function with circulating anti-HLA	13
median 5 years	Stable graft function - future loss of function	20
1-yr	Normal histology	11
1-yr	Calcineurin Inhibitor toxicity	4
1-yr	Interstitial fibrosis and tubular atrophy	15
1-yr	Mixed lesions	14

EXAMPLE 2. Diagnosis/prognosis of the rejection state of included patients using the method according to the invention using a 45-gene signature

The pangenomic nature of the microarrays made it possible to probe the samples for the expression of virtually all genes of the genome without any a priori as to which genes may be of interest.

Once the samples had been run on the microarrays, statistical analyses were performed to identify the individual genes whose expression significantly varied between the two groups as well as an optimal minimum subset of genes that together best discriminated between the group of interest and the controls. The following statistical analysis has been performed : Identification of all differentially expressed genes between the two groups ABMR and Controls. The selection criteria used was a significant t-test of at least 10E-6 and a fold change (in either direction) above 1.5. The identification of an optimal subgroup of genes that together best discriminate between the ABMR cases and the controls was then performed. An ANOVA was used to identify the optimal subset of genes.

In order to minimize the risk of developing a genetic signature based more on a statistical artifact rather than on biologically relevant genes, a parallel analysis was performed based on genes/molecules identified through what is referred to as Intelligent Data Mining. This approach identified genes/molecules considered of relevance to the group of interest (ABMR) from within the literature, public databases and TcLand Expression's in-house data where blood samples from the same group of interest had

already been analyzed by proteomics. For this purpose, the expression of the candidates thus identified was analyzed statistically within the microarray dataset.

These two types of analyses resulted in an integrated list of 135 unique gene candidates. The next step was to transfer the gene signature from the microarray platform to a quantitative PCR platform. For this purpose, the primers and probes corresponding to the 135 genes previously identified were first validated in terms of efficacy and specificity, leading to the removal of 17% of the genes. Among the remaining 112 genes, 60 came from the statistical analysis of the gene expression data obtained by microarray (22 from an optimal multigene discrimination (*Predictive Analysis of Microarray* (PAM) package -like), 38 individually expressed genes) and 52 came from the Intelligent Data Mining step. The 112 gene candidates were analyzed by qPCR in the same samples used for the microarray analysis. Using these data, a fisher linear discrimination was used to predict the group status. To ensure robustness, a leave one out approach was applied when building the predictor. This statistical model is equivalent to a linear model without interactions. This analysis led to the identification of a set of 45 genes.

The group of 45 genes according to the invention was able to correctly classify 77% of the samples analyzed. This yielded sensitivity and negative predictive value for rejection of 79% and 96%, respectively. The AUC is 83%.

These results show that the 45 genes signature according to the invention is useful for diagnosing or prognosing rejection in kidney transplant recipients. Indeed, while not all rejection patients are predicted using this signature (sensitivity=79%), the test is very powerful, so that there is only a very low risk that a patient actually clinically non rejecting is identified, using the 45-gene signature, as a chronic rejecting patient (NPV=96%).

EXAMPLE 3. Identification of subsets of genes able to classify the patients with a good accuracy through an analysis of the pathways

Pathway analysis was performed using Ingenuity Pathway Analysis software (https://analysis.ingenuity.com). The Ingenuity Pathways Analysis application leverages the Ingenuity Pathways Knowledge Base (IPKB) to provide insights into observed gene expression changes across biological samples. Ingenuity dynamically computes a large

"global" molecular network based on hundreds of thousands of curate direct and indirect physical and functional interactions between orthologous mammalian genes from the published, peer-reviewed content in Ingenuity's Knowledge Base. Genes of interest that are also known to directly (or indirectly) interact with other genes in the IPKB, are called Network Eligible Genes and are used to identify molecular networks that indicate how these genes may influence each other.

Ingenuity Pathway Analysis (IPA) identified, among the 45-gene set, 2 networks (see Figures 1 and 2) eligible genes respectively composed of 15 (Table 3) and 14 (Table 4) genes out of the 45 genes according to the invention.

The group of 15 genes according to the invention was able to correctly classify 74,6% of the samples analyzed. This yielded sensitivity and negative predictive value for rejection of 74% and 95%, respectively.

The group of 14 genes according to the invention was able to correctly classify respectively 76,8% of the samples analyzed. This yielded sensitivity and negative predictive value for rejection of 68% and 94%, respectively.

The combination of the 15 and 14 genes, leading to a subset of 29 genes according to the invention, was able to correctly classify 76% of the samples analyzed. This yielded sensitivity and negative predictive value for rejection of 79% and 96%, respectively.

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CLAIMS

1. A method for the in vitro diagnosis of a kidney graft rejection or non-rejection phenotype, comprising:

(a) determining from a kidney grafted subject biological sample an expression profile comprising the 45 genes of Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4,

(b) comparing the obtained expression profile with at least one reference expression profile, and

(c) determining the graft rejection or non-rejection phenotype from said comparison.

2. The method of claim 1, wherein the obtained expression profile is compared to at least one reference kidney graft rejection and/or non-rejection expression profile in step (b).

3. The method of claim 2, wherein the obtained expression profile is compared in step (b) to at least one reference kidney graft rejection expression profile and at least one reference kidney graft non-rejection expression profile.

4. The method of claim 1, wherein the expression profile is determined by measuring the amount of nucleic acid transcripts of said gene(s).

5. The method of claim 4, wherein the expression profile is determined using quantitative PCR or an oligonucleotide microarray.

6. The method of claim 1, wherein the expression profile is determined using a genomic microarray or a proteic microarray.

7. The method according to claim 1, wherein said biological sample is a blood sample.

8. The method according to any of claims 1-7, further comprising determining at least one additional parameter selected from standard biological parameters specific for said subject grafted organ type, phenotypic analyses of peripheral blood cells, and qualitative and/or quantitative analysis of peripheral blood cells immune repertoire.

9. A kit for the in vitro diagnosis of a graft rejection or non rejection phenotype, comprising at least one reagent for the determination of an expression profile comprising the 45 genes of Table 1.

10. The kit of claim 9, further comprising at least one reagent for determining at least one additional parameter selected from standard biological parameters specific for said subject grafted organ type, phenotypic analyses of peripheral blood cells, and qualitative and/or quantitative analysis of the immune repertoire of peripheral blood cells.

11. A nucleic acid microarray comprising nucleic acids specific for the 45 genes of Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4.

12. The nucleic acid microarray according to claim 11, which is an oligonucleotide microarray.

13. A method for designing a kidney transplant recipient immunosuppressive treatment, said method comprising:

(a) determining from a biological sample of said kidney transplant recipient an expression profile comprising or consisting of the 45 genes of following Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4 or equivalent thereof,

(b) Comparing the obtained expression profile with at least one reference expression profile,

(c) Determining the graft rejection or graft non-rejection phenotype of said kidney transplant recipient from said comparison, and

(d) Designing a dose of immunosuppressive drug treatment according to the said identified graft rejection or graft non-rejection phenotype.

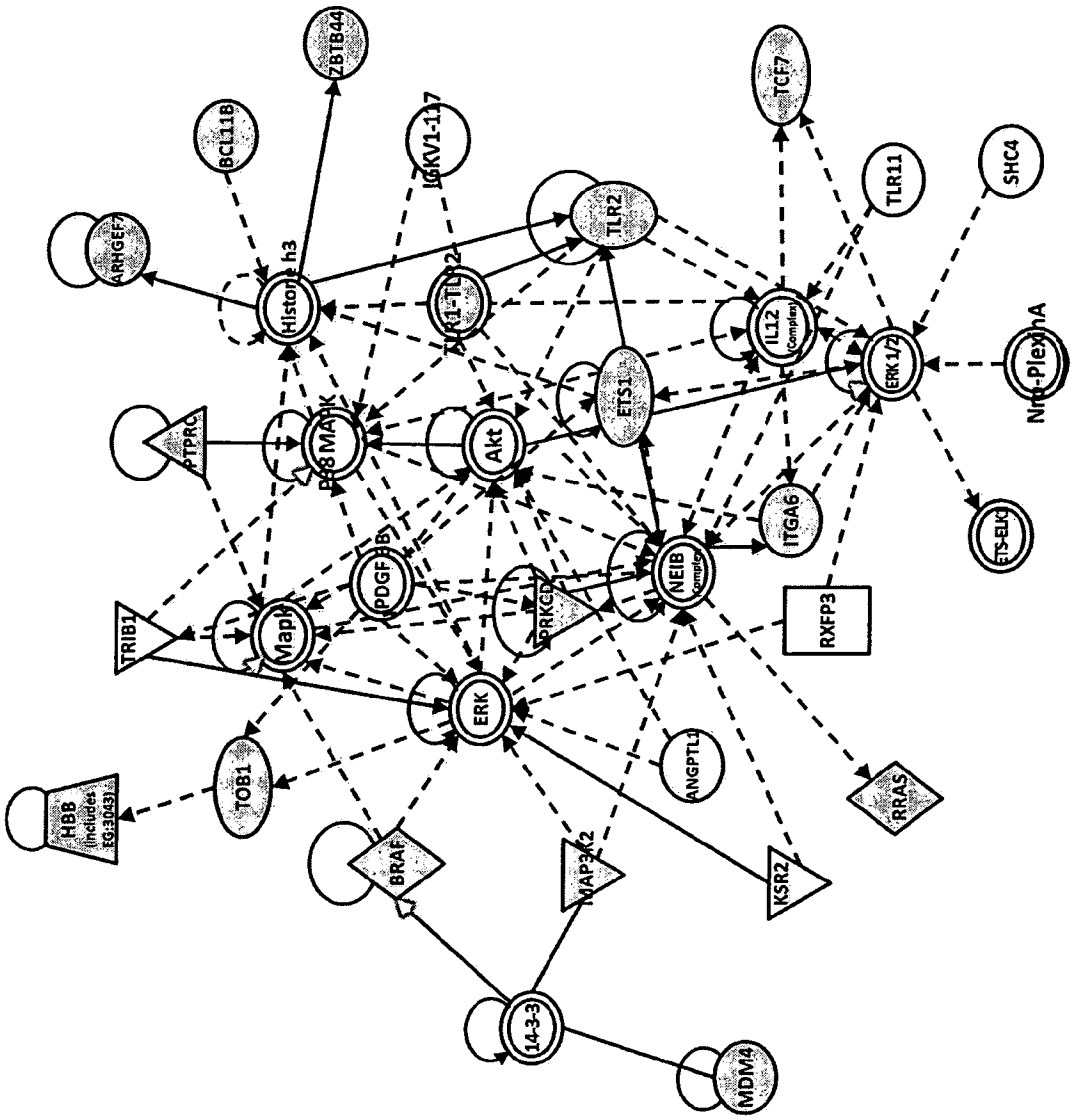
14. A method for adapting the immunosuppressive treatment of a kidney transplant recipient, said method comprising:

(a) Determining from a biological sample of said kidney transplant recipient an expression profile comprising or consisting of the 45 genes of following Table 1 or subsets of these 45 genes such as those described in Tables 2, 3 and 4 or equivalent thereof,

(b) Comparing the obtained expression profile with at least one reference expression profile,

(c) Determining the graft rejection or graft non-rejection phenotype of said kidney transplant recipient from said comparison, and

(d) Adapting the immunosuppressive treatment.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TCF7
TLR11
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Histone h3
TLR2
TLR1-TLR2
IL12 (Complex)
ERK 1/2
Nrp-PlexinA
PTPRC
p38 MAPK
Akt
ETS1
ITGA6
ETS-ELK3
PDGF BB
NEIB (complex)
TRIB1
MapK
PRKCD
RXFP3
ERK
HBB (includes EG:3043)
TOB1
ANGPTL1
RRAS
BRAF
MAP3K2
KSR2
14-3-3
MDM4

Figure 1

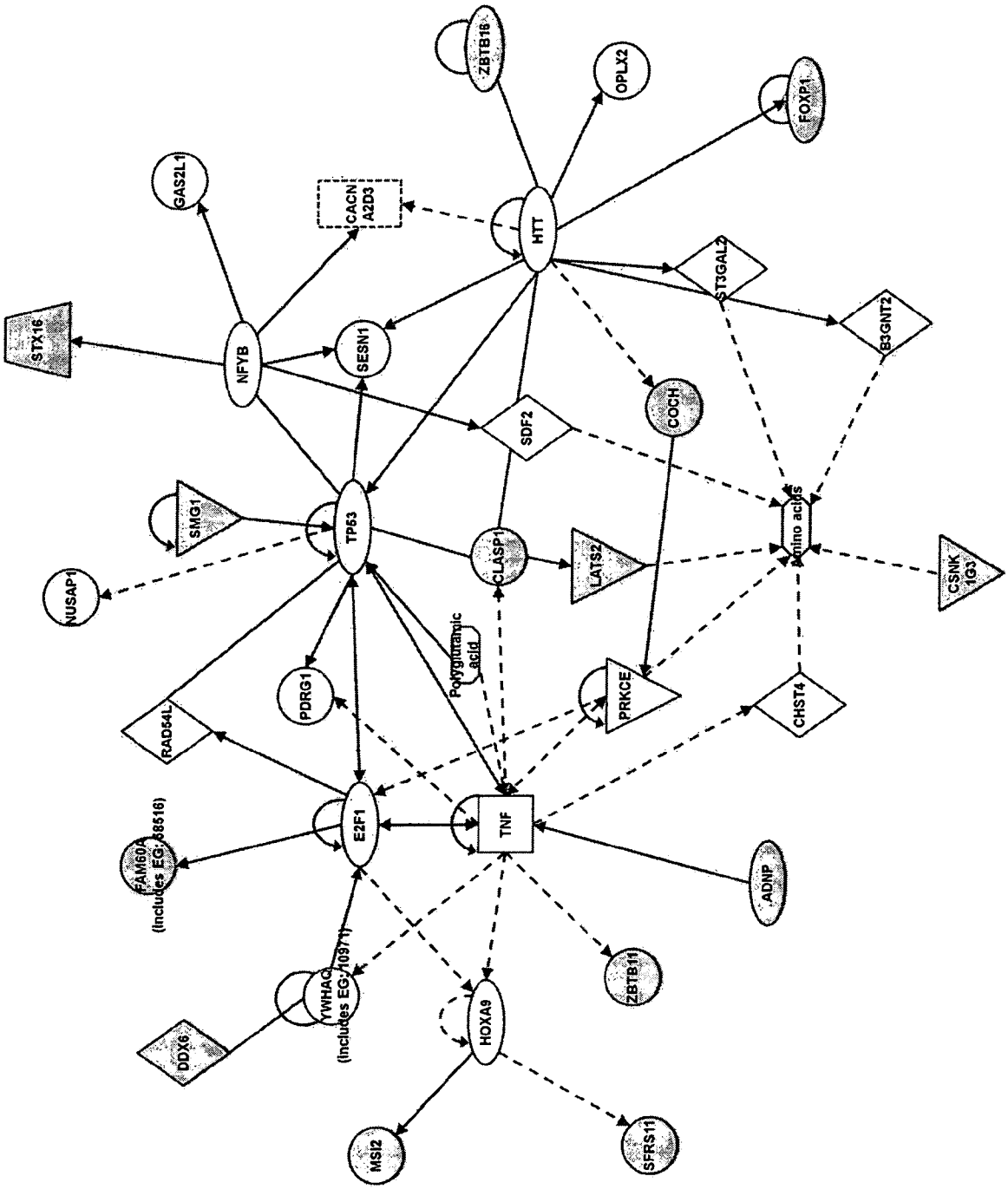
ZBTB16
OPLX2
FOXP1
GAS2L1
CACN A2D3
HTT
ST3GAL2
B3GNT2
STX16
NFYB
SESN1
SDF2
COCH
SMG1
TP53
CLASP1
LATS2
Amino acids
CSNK 1G3
NUSAP1
PDRG1
Polyglutamic acid
PRKCE
CHST4
RAD54L
E2F1
TNF
FAM60A (Includes EG:88516)
ADNP
YWHAG (Includes EG:10971)
ZBTB11
DDX6
HOXA9
MSI2
SFRS11

Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/058126

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

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X	DATABASE Geneseq [Online] 5 February 2009 (2009-02-05), "Human DEAD box polypeptide 6 cDNA, SEQ: 822." XP002603272 retrieved from EBI accession no. GSN:ATZ64669 Database accession no. ATZ64669	9
Y	abstract; compound	10
X	& WO 2008/148115 A1 (ORE PHARMACEUTICALS INC [US]; BIGWOOD DOUGLAS [US]; EASTMAN ERIC [US];) 4 December 2008 (2008-12-04)	9

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2010	23/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Schmitt, Anja

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/058126

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