RISK ASSESSMENT FOR ADVERSE DRUG REACTIONS

FIELD OF THE INVENTION

[0001] This invention relates to methods for predicting the risk of an individual for adverse drug reactions.

REFERENCES

[0002] U.S. Patent No. 6,583,139.

[0003] Arellano, F., and Sacristan, J.A. (1993). Allopurinol hypersensitivity syndrome: a review. Ann. Pharmacother. 27:337.

[0004] Bigby, M., et al. (1986). Drug-induced cutaneous reactions. A report from the Boston Collaborative Drug Surveillance Program on 15,438 consecutive inpatients, 1975 to 1982. JAMA. 256(24):3358-3363.

[0005] Chan, S.H., and Tan, T. (1989). HLA and allopurinol drug eruption. Dermatologica. 79(l):32-33.

[0006] Edwards, S.G., et al. (1999). Concordance of primary generalised epilepsy and carbamazepine hypersensitivity in monozygotic twins. Postgrad Med J. 75(889):680-681.

[0007] Feltkamp, T.E., et al. (2001). Spondyloarthropathies in eastern Asia. Curr Opin Rheumatol. 13(4):285-290.

[0008] Gennis, M.A., et al. (1991). Familial occurrence of hypersensitivity to phenytoin. Am J Med. 91(6):631-634.

[0009] Green, V.J., et al. (1995). Genetic analysis of microsomal epoxide hydrolase in patients with carbamazepine hypersensitivity. Biochem Pharmacol. 50(9):1353-1359.

[0010] Hari, Y., et al. (2001). T cell involvement in cutaneous drug eruptions. Clin Exp Allergy. 31(9):1398-1408.

[0011] IHWG Technical Manual. Genomic Analysis of the Human MHC. DNABased Typing for HLA Alleles and Linked Polymorphisms. Marcel G.J. Tilanus, Editor in Chief. Distributed by the International Histocompatibility Working Group, ISBN number: 0-945278-02-0.

[0012] Khan, M.A. (2000). Update: the twenty subtypes of HLA-B27. Curr Opin Rheumatol. 12(4):235-238.

[0013] Lazarou J., et al. (1998). Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA. 279(15):1200-1205.

[0014] Leeder, J.S. (1998). Mechanisms of idiosyncratic hypersensitivity reactions to antiepileptic drugs. Epilepsia. 39 Suppl 7:S8-16.

[0015] Naisbitt, D.J., et al. (2003). Hypersensitivity reactions to carbamazepine: characterization of the specificity, phenotype, and cytokine profile of drug-specific T cell clones. Mol Pharmacol. 63(3):732-741.

[0016] Pichler, W.J., et al. (1997). High IL-5 production by human drug-specific T cell clones. Int Arch Allergy Immunol. 113(1-3):177-180.

[0017] Pirmohamed, M., et al. (2001). TNFalpha promoter region gene polymorphisms in carbamazepine-hypersensitive patients. Neurology. 56(7):890-896.

[0018] Roujeau, J.C., et al. (1986). HLA phenotypes and bullous cutaneous reactions to drugs. Tissue Antigens. 28(4):251-254.

[0019] Roujeau, J.C., et al. (1987). Genetic susceptibility to toxic epidermal necrolysis. Arch Dermatol. 123(9):1171-1173.

[0020] Roujeau, J.C. (1994). The spectrum of Stevens-Johnson syndrome and toxic epidermal necrolysis: a clinical classification. J Invest Dermatol. 102(6):28S-30S.

[0021] Roujeau, J.C., and Stern, R.S. (1994). Severe adverse cutaneous reactions to drugs. N Engl J Med. 331(19):1272-1285.

[0022] Roujeau, J.C., et al. (1995). Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. N Engl J Med. 333(24):1600-1607.

[0023] Shirato, S., et al. (1997). Stevens-Johnson syndrome induced by methazolamide treatment. Arch Ophthalmol. 115(4):550-553.

[0024] Svensson, C.K., et al. (2000). Cutaneous drug reactions. Pharmacol Rev. 53(3):357-379.

[0025] Tas, S., and Simonart, T. (2003). Management of drug rash with eosinophilia and systemic symptoms (DRESS syndrome): an update. Dermatology. 206(4):353-356.

[0026] Wolkenstein, P., et al. (1995). A slow acetylator genotype is a risk factor for sulphonamide-induced toxic epidermal necrolysis and Stevens-Johnson syndrome. Pharmacogenetics. 5(4):255-258.

[0027] Yates, C.R., et al. (1997). Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med. 126(8):608-614.

[0028] All of the publications, patents and patent applications cited above or elsewhere in this application are herein incorporated by reference in their entirety to the same extent as if the disclosure of each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0029] Adverse drug reactions (ADRs) are a major clinical problem. According to a widely cited meta-analysis, ADRs were ranked between the fourth and sixth most common cause of death (Lazarou et al., 1998). In particular, potentially serious cutaneous ADRs account for about 2-3% of all hospital admissions (Bigby et al., 1986). Although drug eruptions may be mild to moderate, such as maculopapular rash, erythema multiforme (EM), urticaria, and fixed drug eruption, more severe cutaneous ADRs are life threatening and frequently result in death, such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN; Lyell's syndrome), and hypersensitivity syndrome (HSS).

[0030] SJS is characterized by high fever, malaise, and a rapidly developing blistering exanthema of macules and target-like lesions accompanied by mucosal involvement. TEN has similar presentations with an even more extensive skin detachment and a higher mortality rate (30 to 40%). Hypersensitivity Syndrome (HSS) is characterized by skin rash, fever, lymph node enlargement and internal organ involvement; it is also known as DRESS (Drug reaction with eosinophilia and systemic symptoms) or DIHS (drug-induced hypersensitivity syndrome) (Tas and Simonart, 2003). The HSS patients with a wide-spread skin eruption often progress to exfoliative dermatitis, involvement of one or several internal organs (*e.g.*, hepatitis, pneumonitis, nephritis, myocarditis, pericarditis, myositis, pancreatits, thyroiditis), and hematologic abnormalities (eosinophilia, atypical lymphocytosis, neutrophilia, neutropenia, thrombopenia, anemia). Other findings may be lymphadenopathy, periorbital or facial edema. Although the incidence of SJS/TEN is rare with an annual estimated incidence of 3-5 per million people, these conditions can kill or severely disable previously otherwise healthy people (Roujeau and Stern, 1994). The severity of the condition has prompted pharmaceutical companies to withdraw a few newly released drugs.

Almost all SJS/TEN/HSS cases are caused by drugs, most commonly sulfonamides, anticonvulsants, allopurinol, nonsteroidal anti-inflammatory drugs (NSAIDs), and antimalarials (Roujeau et al., 1995). In Taiwan, anticonvulsants (carbamazepine, phenytoin and phenobarbital), and allopurinol are the most common drugs causing SJS/TEN. Other medications such as NSAID and antibiotics are also noted to cause severe ADR.

Recent developments of pharmacogenomics have implied that the susceptibility to ADRs is associated with genetic variants. A successful example of the application of pharmacogenomic study to prevent drug-induced side effects is genotyping thiopurine methyltransferase (TPMT) before prescribing azathioprine, a drug for rheumatologic or cancer diseases (Yates et al, 1997). An individual's genomic polymorphism(s) of TPMT can cause enzyme deficiency and slow metabolizing rate, resulting in leukocytopenia. This kind of molecular diagnostics certified by CLIA (Clinical Laboratory Improvement Amendments) is now offered by reference laboratories in the USA (Prometheus Laboratory Inc.; Genaissance Pharmaceutical) and Europe. Although the susceptibility to SJS/TEN/HSS on certain drugs is thought to be genetically determined (Gennis MA, 1991; Edwards SG, 1999), the responsible genetic factors have yet to be identified, and currently there is no method clinically useful that can be used to predict who will develop SJS/TEN/HSS or to which drugs.

SUMMARY OF THE INVENTION

Disclosed herein is a method of predicting the risk of a patient for developing adverse drug reaction, particularly SJS, Ten, or hypersensitivity syndrome (HSS). It was discovered that an HLA-B allele, HLA-B*1502, is associated with SJS/TEN that is induced by a variety of drugs. The correlation with HLA-B*1502 is most significant for carbamazepine-induced SJS/TEN, wherein all the patients tested have the HLA-B*1502 allele. In addition, another HLA-B allele, HLA-B*5801, is particularly associated with SJS/TEN induced by allopurinol. HLA-B*5801 is also associated with allopurinol-induced hypersensitivity syndrome (HSS). Milder cutaneous reactions induced by carbamazepine, such as maculopapular rash, erythema multiforme (EM), urticaria, and fixed drug eruption, are particularly associated with a third allele, HLA-B*4601.

Accordingly, also disclosed is a method of assessing the risk of a patient for developing an adverse drug reaction in response to a drug, comprising performing HLA typing using a biological sample from the patient. Any HLA allele that is associated with the ADR with a sensitivity of at least about 40% can be used as the risk factor in the present invention. Preferably, the sensitivity of the risk factor is at least about 50%, 60%, 70%, 80%, 85% or 90%. More preferably, the sensitivity is at least 95%. The drug is preferably selected from the group consisting of carbamazepine, allopurinol, phenytoin, sulfasalazine, amoxicillin, ibuporfen and ketoprofen. Alternatively, the drug is preferably not a nonsteroidal anti-inflammatory drug. Preferably, an HLA-B allele is the risk factor.

Specifically, disclosed herein is a method of assessing the risk of a patient for developing an adverse drug reaction in response to a drug, comprising determining the presence of an HLA-B allele selected from the group consisting of HLA-B*1502, HLA-B*5801 and HLA-B*4601, wherein the presence of the HLA-B allele is indicative of a risk for an adverse drug reaction. The drug is preferably selected from the group consisting of carbamazepine, allopurinol, phenytoin, sulfasalazine, amoxicillin, ibuporfen and ketoprofen. Most preferably, the drug is carbamazepine, allopurinol or phenytoin.

The adverse drug reaction is preferably a cutaneous adverse drug reaction, such as Stevens-Johnson syndrome, toxic epidermal necrolysis or HSS. In a preferred embodiment, the drug is carbamazepine, and the allele is HLA-B*1502. In another preferred embodiment, the allele HLA-B*5801 is used to predict the risk for cutaneous ADR, such as Stevens-Johnson syndrome or toxic epidermal necrolysis in response to allopurinol. HLA-B*5801 is also a marker for allopurinol-induced drug reaction with eosinophilia and systemic symptoms, such as allopurinol-induced hypersensitivity syndrome. Other subtypes of HLA-B15, B58 or B46 can also be used to predict the risk for ADR instead of HLA-B*1502, HLA-B*5801 or HLA-B*4601, such as HLA-B*1503 or *1558.

The allele can be detected by using any method known in the art. For example, the presence of the allele can be determined by using an oligonucleotide that specifically hybridizes with the nucleic acid coding for the allele. Preferably, the DNA prepared from the peripheral blood of the patient is employed in the determination. The allele can also be detected by, for example, serological or microcytotoxicity methods.

The presence of the allele of interest can also be determined by detecting an equivalent genetic marker of the allele, which is a genetic marker that is linked to the allele. An equivalent genetic maker can be, *e.g.,* an SNP (single nucleotide polymorphism), microsatellite marker or any kind of genetic polymorphism. For example, the HLA-markers of HLA-B B*1502 haplotype include, without being limited to, DRB1*1202, Cw*0801, Cw*0806, A*1101, and MICA*019. The HLA-markers of the HLA-B*5801 haplotype comprise, for example, HLA-A*3303, Cw*0302, DRB1*0301, and MICA*00201. In other words, the presence of the HLA-B*1502, 5801 or 4601 haplotype, rather than the alleles *per se,* is indicative of a risk for adverse drug reactions.

Also disclosed is a method of pharmacogenomics profiling comprising determining the presence of at least one HLA-B allele selected from the group consisting of HLA-B*1502, HLA-B*5801, and HLA-B*4601. Preferably, the presence of at least two alleles selected from the group is determined, such as HLA-B*1502 and HLA-B*5801. More preferably, the presence of all three alleles is determined. The method can optionally comprise the determination of other generic factors. Those other genetic factors may be associated with the predisposition for any disease or medical condition, including adverse drug reactions. For example, these other genetic factors maybe selected from the group consisting of thiopurine methyltransferase and the genes for the long-QT syndrome.

Further provided is a method of screening and/or identifying medicines that can be used to treat drug-induced SJS/TEN, and/or HSS by using HLA-B*1502, 5801 or 4601 as a target in drug development. For example, cells expressing any of the alleles can be contacted with medicine candidates, and the candidates that binds to the allele are likely to inhibit the function of the allele. The efficacy of the allele-binding candidate in treating drug induced reactions can then be further tested.

In addition, an HLA allele that is associated with SJS/TEN/HSS can also be used to screen for drugs that induce SJS/TEN/HSS. For example, a candidate drug may be contacted with the HLA allele, and the candidate that bind to the allele may induce SJS/TEN/HSS and can be further tested. Cells or animals that comprise the HLA allele can also be used to screen for drugs that induce SJS/TEN/HSS.

In one aspect of the present invention, there is provided an *in-vitro* method of assessing the risk of a patient for developing an adverse drug reaction in response to a drug, comprising determining from a biological sample, the presence of an HLA-B allele selected from the group consisting of HLA-B* 1502, HLA-B* 5801 and HLA-B*4601, wherein the presence of the HLA-B allele is indicative of a risk for an adverse drug reaction.

In another aspect of the present invention, there is provided an *in-vitro* method for developing a therapy for an adverse reaction induced by a drug, comprising screening candidate medicines using an *in-vitro* assay in which at least one HLA-B allele is a target, wherein the HLA-B allele is selected from the group consisting of HLA-B* 1502, HLA-B*5801, and HLA-B*4601.

In another aspect of the present invention, there is provided an *in-vitro* method of pharmacogenomics profiling comprising determining from a biological sample, the presence of at least two HLA-B allele selected from the group consisting of HLA-B*1502, HLA-B*5801,and HLA-B*4601.

In another aspect of the present invention, there is provided an *in-vitro* method for identifying a drug that induces SJS, TEN or HSS, comprising contacting, *in-vitro,* a cell that comprises an HLA subtype with a candidate drug, wherein the HLA subtype is an HLA-B*1502, HLA-B*5801 or HLA-B*4601.

DETAILED DESCRIPTION OF THE INVENTION

[0042] The present invention provides a method of predicting the risk of a patient for developing adverse drug reactions, particularly SJS, TEN, or HSS. It was discovered that an HLA-B allele, HLA-B*1502, is associated with SJS/TEN that is induced by a variety of drugs. The correlation with HLA-B*1502 is most significant for carbamazepine-induced SJS/TEN, wherein all the patients tested have the HLA-B*1502 allele. In addition, another HLA-B allele, HLA-B*5801, is particularly associated with SJS/TEN or HSS induced by allopurinol. Milder cutaneous reactions associated with carbamazepine, such as maculopapular rash, erythema multiforme (EM), urticaria, and fixed drug eruption, are particularly associated with a third allele, HLA-B*4601.

[0043] Prior to describing the invention in further detail, the terms used in this application are defined as follows unless otherwise indicated.

Definitions

[0044] An "adverse drug reaction" is an undesired and unintended effect of a drug. In particular, an adverse drug reaction occurs at doses used for prophylaxis, diagnosis or therapy.

[0045] A "drug", or "medication", is any compound or material that is administered to a patient for prophylactic, diagnostic or therapeutic purposes.

[0046] A patient has a "risk" for an adverse drug reaction if the probability of the patient to develop an adverse drug reaction is higher than the probability of the general population to develop the adverse drug reaction. The probability of the patient to develop the adverse drug reaction is preferably at least about 1.5 fold, more preferably at least about 2 fold, still more preferably at least about 3, 4, 5, 6, 7, 8 or 9 fold, and most preferably at least about 10 fold as high as the probability of the general population to develop the adverse drug reaction. The probability can be determined by any method known in the art, such as by using the incidence of risk factors. For example, a given risk factor is present in 5% of the general population. If this factor is present in 10% of the patients who have an adverse drug reaction, then the probability of a patient with this risk factor to develop the adverse drug reaction is 2 fold as high as the probability of the general population to develop the adverse drug reaction.

[0047] A "risk factor" for an ADR is a factor that is associated with the ADR. The sensitivity of a risk factor is preferably at least about 40%, more preferably at least about 50%, 60%, 70%, 80%, 85% or 90%. Most preferably, the sensitivity is at least 95%.

[0048] The "sensitivity" of a risk factor for predicting an ADR is the percentage of patients with the ADR that possess the risk factor. In other words, if every SJS patient has allele A, the sensitivity of allele A for predicting SJS is 100%. If 20 out of 40 SJS patients have allele B, then the sensitivity of allele B for predicting SJS is 50%.

[0049] An "equivalent genetic marker" of an allele of interest refers to a genetic marker that is linked to the allele of interest. The useful equivalent genetic markers in the present invention display a linkage disequilibrium with the allele of interest.

[0050] "Pharmacogenomics profiling" refers to the determination of genetic factors present in a subject that are associated with diseases or medical conditions, particularly adverse reactions to drugs. Typically, a panel of genetic factors is determined in pharmacogenomics profiling, and the factors may or may not be associated with the same disease, medical condition, or reaction to drug.

[0051] A "metabolite" of a drug refers to a compound that can be derived from the drug due to metabolism in a living organism, preferably a mammal, and more preferably a human.

[0052] A "derivative" of a drug, as used herein, refers to a compound which is the same as the drug except that at least one hydrogen in the drug is substituted with a halo, hydroxyl, acylamino, alkyl, alkenyl, alkynyl, alkoxy, aryloxy, aryl, aryloxyaryl, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxylsubstituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, cycloalkyl, substituted alkyl, substituted alkoxy, substituted aryl, substituted aryloxy, substituted aryloxyaryl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic or substituted heterocyclic group. The chemical groups are defined below or as defined in U.S. Patent No. 6,583,139. The substituent contains preferably zero to ten, more preferably zero to six, more preferably zero to four, and most preferably zero to two carbon atoms.

[0053] As used herein, "alkyl" refers to alkyl groups preferably having from 1 to 10 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups is such as methyl, t-butyl, n-heptyl, octyl and the like.

[0054] "Substituted alkyl" refers to an alkyl group, preferably of from 1 to 10 carbon atoms, having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkyl amidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxylaryl, substituted aryloxyaryl, cyano, halogen, hydroxyl, nitro, carboxyl, carboxyalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxylsubstituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted aryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, $-OS(O)_2$ -alkyl, $-OS(O)_2$ -substituted alkyl, $-OS(O)_2$ -aryl, $-OS(O)_2$ -substituted aryl, $-OS(O)_2$ -heteroaryl, $-OS(O)_2$ -substituted heteroaryl, $-OS(O)_2$ -heterocyclic, $-OS(O)_2$ - substituted heterocyclic, $-OSO_2$ -NRR, $-NRS(O)_2$ -alkyl, $-NRS(O)_2$ -substituted alkyl, $-NRS(O)_2$ -aryl, $-NRS(O)_2$ -substituted aryl, $-NRS(O)_2$ -heteroaryl, $-NRS(O)_2$ -substituted heteroaryl, $-NRS(O)_2$ -heterocyclic, $-NRS(O)_2$ -substituted heterocyclic, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted alkyl, $-NRS(O)_2$ -NR-aryl, $-NRS(O)_2$ -NR-substituted aryl, $-NRS(O)_2$ -NR-heteroaryl, $-NRS(O)_2$ -NR-substituted heteroaryl, $-NRS(O)_2$ -NR-heterocyclic, $-NRS(O)_2$ -NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, monoand di-arylamino, mono- and di-(substituted aryl)amino, mono- and diheteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and diheterocyclic amino, mono- and di-(substituted heterocyclic) amino, unsymmetric disubstituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic; substituted alkyl groups having amino groups blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like) and alkyl/substituted alkyl groups substituted with $-SO_2$ -alkyl, $-SO_2$ -substituted alkyl, $-SO_2$ -

alkenyl, -SO_2 -substituted alkenyl, -SO_2 -cycloalkyl, -SO_2 -substituted cycloalkyl, -SO_2 -aryl, -SO_2 -substituted aryl, -SO_2 -heteroaryl, -SO_2 - substituted heteroaryl, -SO_2 -heterocyclic, -SO_2 -substituted heterocyclic or -SO_2 -NRR, where R is hydrogen or alkyl.

[0055] "Alkoxy" refers to the group "alkyl-O-" which includes, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, npentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

[0056] "Substituted alkoxy" refers to the group "substituted alkyl-O-".

[0057] "Acyl" refers to the groups H-C(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, alkenyl-C(O)-, substituted alkenyl-C(O)-, alkynyl-C(O)-, substituted alkynyl-C(O)cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, aryl-C(O)-, substituted aryl-C(O)-, heteroaryl-C(O)-, substituted heteroaryl-C(O), heterocyclic-C(O)-, and substituted heterocyclic-C(O)- wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0058] "Acylamino" refers to the group -C(O)NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic; and where each R can be joined to form, together with the nitrogen atom, a heterocyclic or substituted heterocyclic ring wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0059] "Acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, alkenyl-C(O)O-, substituted alkenyl-C(O)O-, alkynyl-C(O)O-, substituted alkynylC(O)O-, aryl-C(O)O-, substituted aryl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, heteroaryl-C(O)O-, substituted heteroaryl-C(O)O-, heterocyclic-C(O)O-, and substituted heterocyclic-C(O)O- wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0060] "Alkenyl" refers to alkenyl group preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of alkenyl unsaturation.

[0061] "Substituted alkenyl" refers to alkenyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxylcycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxylsubstituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, $-OS(O)_2$ -alkyl, $-OS(O)_2$ - substituted alkyl, $-OS(O)_2$ -aryl, $-OS(O)_2$ -substituted aryl, $-OS(O)_2$ - heteroaryl, $-OS(O)_2$ - substituted heteroaryl, $-OS(O)_2$ -heterocyclic, $-OS(O)_2$ - substituted heterocyclic, $-OSO_2$ - NRR, $-NRS(O)_2$ -alkyl, $-NRS(O)_2$ -substituted alkyl, $-NRS(O)_2$ -aryl, $-NRS(O)_2$ -substituted aryl, $-NRS(O)_2$ -heteroaryl, $-NRS(O)_2$ -substituted heteroaryl, $-NRS(O)_2$ -heterocyclic, $-NRS(O)_2$ -substituted heterocyclic, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted alkyl, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted aryl, $-NRS(O)_2$ -NR-heteroaryl, $-NRS(O)_2$ -- NR-substituted heteroaryl, $-NRS(O)_2$ -NR-heterocyclic, $-NRS(O)_2$ -NR-substituted heterocyclic; mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, monoand di-arylamino, mono- and di-(substituted aryl)amino, mono- and diheteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and diheterocyclic amino, mono- and di-(substituted heterocyclic) amino, unsymmetric disubstituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic and substituted alkenyl groups having amino groups blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like) and alkenyl/substituted alkenyl groups substituted with $-SO_2$ -alkyl, $-SO_2$ - substituted alkyl, $-SO_2$ -alkenyl, $-SO_2$ -substituted alkenyl, $-SO_2$ -cycloalkyl, $-SO_2$ -substituted cycloalkyl,

$-SO_2$ -aryl, $-SO_2$ -substituted aryl, $-SO_2$ -heteroaryl, $-SO_2$ - substituted heteroaryl, $-SO_2$ - heterocyclic, $-SO_2$ -substituted heterocyclic or $-SO_2$ NRR, where R is hydrogen or alkyl.

[0062] "Alkynyl" refers to alkynyl group preferably having from 2 to 10 carbon atoms and more preferably 3 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of alkynyl unsaturation.

[0063] "Substituted alkynyl" refers to alkynyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, arninocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxylcycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxylsubstituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, $-OS(O)_2$ -alkyl, $-OS(O)_2$ - substituted alkyl, $-OS(O)_2$ -aryl, $-OS(O)_2$ -substituted aryl, $-OS(O)_2$-heteroaryl, $-OS(O)_2$ - substituted heteroaryl, $-OS(O)_2$ -heterocyclic, $-OS(O)_2$ - substituted heterocyclic, $-OSO_2$ - NRR, $-NRS(O)_2$ -alkyl, $-NRS(O)_2$ -substituted alkyl, $-NRS(O)_2$ -aryl, $-NRS(O)_2$ -substituted aryl, $-NRS(O)_2$ -heteroaryl, $-NRS(O)_2$ -substituted heteroaryl, $-NRS(O)_2$ -heterocyclic, - $NRS(O)_2$ -substituted heterocyclic, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted alkyl, - $NRS(O)_2$ -NR-aryl, $-NRS(O)_2$ -NR-substituted aryl, $-NRS(O)_2$ -NR-heteroaryl, $-NRS(O)_2$ -- NR-substituted heteroaryl, $-NRS(O)_2$ -NR-heterocyclic, $-NRS(O)_2$ -NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, monoand di-arylamino, mono- and di-(substituted aryl)amino, mono- and diheteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and diheterocyclic amino, mono- and di-(substituted heterocyclic) amino, unsymmetric disubstituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic; substituted alkynyl groups

having amino groups blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like), and alkynyl/substituted alkynyl groups substituted with -SO_2 -alkyl, -SO_2 -substituted alkyl, -SO_2 -alkenyl, -SO_2 -substituted alkenyl, -SO_2 -cycloalkyl, -SO_2 -substituted cycloalkyl, -SO_2 -aryl, -SO_2 -substituted aryl, -SO_2 -heteroaryl, -SO_2 - substituted heteroaryl, -SO_2 -heterocyclic, -SO_2 -substituted heterocyclic or -SO_2 NRR, where R is hydrogen or alkyl.

[0064] "Aminoacyl" refers to the groups -NRC(O)alkyl, -NRC(O)substituted alkyl, -NRC(O)cycloalkyl, -NRC(O)substituted cycloalkyl, -NRC(O)alkenyl, -NRC(O)substituted alkenyl, -NRC(O)alkynyl, -NRC(O)substituted alkynyl, -NRC(O)aryl, -NRC(O)substituted aryl, -NRC(O)heteroaryl, -NRC(O)substituted heteroaryl, -NRC(O)heterocyclic, and -NRC(O)substituted heterocyclic, where R is hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0065] "Aminocarbonyloxy" refers to the groups -NRC(O)O-alkyl, -NRC(O)O-substituted alkyl, -NRC(O)O-alkenyl, -NRC(O)O-substituted alkenyl, -NRC(O)Oalkynyl, -NRC(O)O-substituted alkynyl, -NRC(O)O-cycloalkyl, -NRC(O)Osubstituted cycloalkyl, -NRC(O)O-aryl, -NRC(O)O-substituted aryl, -NRC(O)Oheteroaryl, -NRC(O)O-substituted heteroaryl, -NRC(O)O-heterocyclic, and -NRC(O)O-substituted heterocyclic where R is hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0066] "Oxycarbonylamino" refers to the groups -OC(O)NRR, -OC(O)NR-alkyl, -OC(O)NR-substituted alkyl, -OC(O)NR-alkenyl, -OC(O)NR-substituent alkenyl, -OC(O)NR-alkynyl, -OC(O)NR-substituted alkynyl, -OC(O)NR-cycloalkyl, -OC(O)NR-substituted cycloalkyl, -OC(O)NR-aryl, -OC(O)NR-substituted aryl, -OC(O)NR-heteroaryl, -OC(O)NR-substituted heteroaryl, -OC(O)NR-heterocyclic, and -OC(O)NR-substituted heterocyclic where R is hydrogen or alkyl, and where each R can be joined to form, together with the nitrogen atom, a heterocyclic or substituted heterocyclic ring and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0067] "Aryl" or "Ar" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (*e.g.*, phenyl) or multiple condensed rings (*e.g.*, naphthyl or anthryl) which condensed rings may or may not be aromatic (*e.g.*, 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7-yl, and the like). Preferred aryls include phenyl and naphthyl.

[0068] Substituted aryl refers to aryl groups which are substituted with from 1 to 3 substituents selected from the group consisting of hydroxy, acyl, acylamino, thiocarbonylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amidino, alkylamidino, thioamidino, amino, aminoacyl, aminocarbonyloxy, aminocarbonylamino, aminothiocarbonylamino, aryl, substituted aryl, aryloxy, substituted aryloxy, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, carboxyl, carboxylalkyl, carboxylsubstituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, carboxylamido, cyano, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thioheteroaryl, substituted thioheteroaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheterocyclic, substituted thioheterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, $-SO(O)_2$ -alkyl, $-S(O)_2$ - substituted alkyl, $-S(O)_2$ -cycloalkyl, $-S(O)_2$ -substituted cycoalkyl, $-S(O)_2$ -alkenyl, $-S(O)_2$ - substituted alkenyl, $-S(O)_2$ -aryl, $-S(O)_2$ -substituted aryl, $-S(O)_2$ -heteroaryl, $-S(O)_2$ - substituted heteroaryl, $-S(O)_2$ -heterocyclic, $-S(O)_2$ -substituted heterocyclic, $-OS(O)_2$ -alkyl, $-OS(O)_2$ -substituted alkyl, $-OS(O)_2$ -aryl, $-OS(O)_2$ -substituted aryl, $-OS(O)_2$ -heteroaryl, $-OS(O)_2$ -substituted heteroaryl, $-OS(O)_2$ -heterocyclic, $-OS(O)_2$ -substituted heterocyclic, $-OSO_2$ -NRR, $-NRS(O)_2$ -alkyl, $-NRS(O)_2$ - substituted alkyl, $-NRS(O)_2$ -aryl, $-NRS(O)_2$ - substituted aryl, $-NRS(O)_2$ - heteroaryl, $-NRS(O)_2$ -substituted heteroaryl, $-NRS(O)_2$ - heterocyclic, $-NRS(O)_2$ - substituted heterocyclic, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted alkyl, $-NRS(O)_2$ -NR-aryl, $-NRS(O)_2$ -NR-substituted aryl, $-NRS(O)_2$ -NR-heteroaryl, $-NRS(O)_2$ -NR-substituted heteroaryl, $-NRS(O)_2$ -NR-substituted $-NRS(O)_2$ -NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-(substituted aryl)amino, monoand di-heteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and diheterocyclic amino, mono- and di-

(substituted heterocyclic) amino, unsymmetric disubstituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, amino groups on the substituted aryl blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like), and -SO_2 NRR, where R is hydrogen or alkyl.

[0069] "Aryloxy" refers to the group aryl-O- which includes, by way of example, phenoxy, naphthoxy, and the like.

[0070] "Substituted aryloxy" refers to substituted aryl-O- groups.

[0071] "Aryloxyaryl" refers to the group -aryl-O-aryl.

[0072] "Substituted aryloxyaryl" refers to aryloxyaryl groups substituted with from 1 to 3 substituents on either or both aryl rings selected from the group consisting of hydroxy, acyl, acylamino, thiocarbonylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amidino, alkylamidino, thioamidino, amino, aminoacyl, aminocarbonyloxy, aminocarbonylamino, aminothiocarbonylamino, aryl, substituted aryl, aryloxy, substituted aryloxy, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, carboxylamido, cyano, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thioheteroaryl, substituted thioheteroaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheterocyclic, substituted thioheterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -$S(O)_2$ -alkyl, -$S(O)_2$ - substituted alkyl, -$S(O)_2$ -cycloalkyl, -$S(O)_2$ -substituted cycloalkyl, -$S(O)_2$ - alkenyl, -$S(O)_2$ -substituted alkenyl, -$S(O)_2$ -aryl, -$S(O)_2$ -substituted aryl, -$S(O)_2$ - heteroaryl, -$S(O)_2$ -substituted heteroaryl, -$S(O)_2$ -heterocyclic, -$S(O)_2$ -substituted heterocyclic, -$OS(O)_2$ -alkyl, -$OS(O)_2$ -substituted alkyl, -$OS(O)_2$ -aryl, -$OS(O)_2$ - substituted aryl, -$OS(O)_2$ -heteroaryl, -$OS(O)_2$ -substituted heteroaryl, -$OS(O)_2$ - heterocyclic, -$OS(O)_2$ - substituted heterocyclic, -OSO_2 -NRR, -$NRS(O)_2$ -alkyl, -$NRS(O)_2$ -substituted alkyl, -

$NRS(O)_2$ -aryl, $-NRS(O)_2$ -substituted aryl, $-NRS(O)_2$ -heteroaryl, $-NRS(O)_2$ -substituted heteroaryl, $-NRS(O)_2$ -heterocyclic, $-NRS(O)_2$ - substituted heterocyclic, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted alkyl, $-NRS(O)_2$ -NR-aryl, $-NRS(O)_2$ -NR-substituted aryl, $-NRS(O)_2$ -NR-heteroaryl, $-NRS(O)_2$ -NR-substituted heteroaryl, $-NRS(O)_2$ -NR-heterocyclic, $-NRS(O)_2$ -NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-(substituted aryl)amino, monoand di-heteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and diheterocyclic amino, mono- and dl-(substituted heterocyclic) amino, unsymmetric disubstituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic, amino groups on the substituted aryl blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like) and substituted with $-SO_2$ NRR, where R is hydrogen or alkyl.

[0073] "Cycloalkyl" refers to cyclic alkyl groups of from 3 to 8 carbon atoms having a single cyclic ring including, by way of example, cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl and the like. Excluded from this definition are multi-ring alkyl groups such as adamantanyl, etc.

[0074] "Cycloalkenyl" refers to cyclic alkenyl groups of from 3 to 8 carbon atoms having single or multiple unsaturation but which are not aromatic.

[0075] "Substituted-cycloalkyl" and "substituted cycloalkenyl" refer to a cycloalkyl and cycloalkenyl groups, preferably of from 3 to 8 carbon atoms, having from 1 to 5 substituents selected from the group consisting of oxo (=O), thioxo (=S), alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxylsubstituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy,

substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -$OS(O)_2$ -alkyl, -$OS(O)_2$ - substituted alkyl, -$OS(O)_2$ -aryl, -$OS(O)_2$ -substituted aryl, -$OS(O)_2$-heteroaryl, -$OS(O)_2$ - substituted heteroaryl, -$OS(O)_2$ -heterocyclic, -$OS(O)_2$-substituted heterocyclic, -OSO_2 -NRR, -$NRS(O)_2$ -alkyl, -$NRS(O)_2$ –substituted alkyl, -$NRS(O)_2$ -aryl, -$NRS(O)_2$ -substituted aryl, -$NRS(O)_2$ -heteroaryl, -$NRS(O)_2$ -substituted heteroaryl, -$NRS(O)_2$ -heterocyclic, -$NRS(O)_2$ – substituted heterocyclic, -$NRS(O)_2$ -NR-alkyl, -$NRS(O)_2$ -NR-substituted alkyl, -$NRS(O)_2$ -- NR-aryl, -$NRS(O)_2$ -NR-substituted aryl, -$NRS(O)_2$ -NR-heteroaryl, -$NRS(O)_2$-NR-substituted heteroaryl, -$NRS(O)_2$ -NR-heterocyclic, -$NRS(O)_2$ -NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, monoand di-arylamino, mono- and di-(substituted aryl)amino, mono- and di-heteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and di-heterocyclic amino, mono- and di-(substituted heterocyclic) amino, unsymmetric di-substituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic, substituted alkynyl groups having amino groups blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like) and alkynyl/substituted alkynyl groups substituted with -SO_2 -alkyl, -SO_2 - substituted alkyl, -SO_2 -alkenyl, -SO_2 -substituted alkenyl, -SO_2 -cycloalkyl, -SO_2 -substituted cycloalkyl, -SO_2 -aryl, -SO_2 -substituted aryl, -SO_2 -heteroaryl, -SO_2 - substituted heteroaryl, -SO_2 -heterocyclic, -SO_2 -substituted heterocyclic or -SO_2 NRR, where R is hydrogen or alkyl.

[0076] "Cycloalkoxy" refers to -O-cycloalkyl groups.

[0077] "Substituted cycloalkoxy" refers to -O-substituted cycloalkyl groups.

[0078] "Halo" refers to fluoro, chloro, bromo and iodo and preferably is either chloro or bromo.

[0079] "Heteroaryl" refers to an aromatic carbocyclic group of from 2 to 10 carbon atoms and 1 to 4 heteroatoms selected from the groups consisting of oxygen, nitrogen and sulfur within the ring. Such heteroaryl groups can have a single ring (*e.g.*, pyridyl or furyl) or multiple condensed rings (*e.g.*, indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl, indolyl and furyl.

[0080] "Substituted heteroaryl" refers to heteroaryl groups which are substituted with from 1 to 3 substituents selected from the group consisting of hydroxy, acyl, acylamino, thiocarbonylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amidino, alkylamidino, thioamidino, amino, aminoacyl, aminocarbonyloxy, aminocarbonylamino, aminothiocarbonylamino, aryl, substituted aryl, aryloxy, substituted aryloxy, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, carboxylamido, cyano, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thioheteroaryl, substituted thioheteroaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheterocyclic, substituted thioheterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, $-S(O)_2$ -alkyl, $-S(O)_2$ - substituted alkyl, $-S(O)_2$ -cycloalkyl, $-S(O)_2$ -substituted cycloalkyl, $-S(O)_2$ - alkenyl, $-S(O)_2$ -substituted alkenyl, $-S(O)_2$ -aryl, $-S(O)_2$ -substituted aryl, $-S(O)_2$ - heteroaryl, $-S(O)_2$ - substituted heteroaryl, $-S(O)_2$ -heterocyclic, $-S(O)_2$ -substituted heterocyclic, $-OS(O)_2$ -alkyl, $-OS(O)_2$ -substituted alkyl, $-OS(O)_2$ -aryl, $-OS(O)_2$ - substituted aryl, $-OS(O)_2$ -heteroaryl, $-OS(O)_2$ -substituted heteroaryl, $-OS(O)_2$ - heterocyclic, $-OS(O)_2$ -substituted heterocyclic, $-OSO_2$ -NRR, $-NRS(O)_2$ -alkyl, $-NRS(O)_2$ -substituted alkyl, $-NRS(O)_2$ -aryl, $-NRS(O)_2$ - substituted aryl, $-NRS(O)_2$ -heteroaryl, $-NRS(O)_2$ -substituted heteroaryl, $-NRS(O)_2$ - heterocyclic, $-NRS(O)_2$ - substituted heterocyclic, $-NRS(O)_2$ -NR-alkyl, $-NRS(O)_2$ -NR-substituted alkyl, $-NRS(O)_2$ -NR-aryl, $-NRS(O)_2$ -NR-substituted aryl, $-NRS(O)_2$ -NR-heteroaryl, $-NRS(O)_2$ -NR-substituted heteroaryl, $-NRS(O)_2$ -NR-heterocyclic, $-NRS(O)_2$ -- NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-(substituted aryl)amino, mono- and di-heteroarylamino, mono- and di-(substituted heteroaryl)amino, mono- and di-heterocyclic amino, mono- and di-(substituted heterocyclic) amino, unsymmetric di-substituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted

heterocyclic, amino groups on the substituted aryl blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like), and -SO_2 NRR, where R is hydrogen or alkyl.

[0081] "Heteroaryloxy" refers to the group -O-heteroaryl and "substituted heteroaryloxy" refers to the group -O-substituted heteroaryl.

[0082] "Heterocycle" or "heterocyclic" refers to a saturated or unsaturated group having a single ring or multiple condensed rings, containing from 1 to 10 carbon atoms and from 1 to 4 heteroatoms selected from the group consisting of nitrogen, sulfur or oxygen within the ring. In fused ring systems, one or more of the rings can be aryl or heteroaryl.

[0083] "Substituted heterocyclic" refers to heterocycle groups which are substituted with from 1 to 3 substituents selected from the group consisting of oxo (=O), thioxo (=S), alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloakyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxylsubstituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -$OS(O)_2$ -alkyl, -$OS(O)_2$ -substituted alkyl, -$OS(O)_2$ -aryl, -$OS(O)_2$ -substituted aryl, -$OS(O)_2$-heteroaryl, -$OS(O)_2$ -substituted heteroaryl, -$OS(O)_2$ -heterocyclic, -$OS(O)_2$-substituted heterocyclic, -OSO_2 -NRR, -$NRS(O)_2$ -alkyl, -$NRS(O)_2$ –substituted alkyl, -$NRS(O)_2$ -aryl, -$NRS(O)_2$ -substituted aryl, -$NRS(O)_2$ -heteroaryl, -$NRS(O)_2$ -substituted heteroaryl, -$NRS(O)_2$ -heterocyclic, -$NRS(O)_2$ –substituted heterocyclic, -$NRS(O)_2$ -NR-alkyl, -$NRS(O)_2$ -NR-substituted alkyl, -$NRS(O)_2$ -NR-alkyl, -$NRS(O)_2$ -NR-substituted aryl, -$NRS(O)_2$ -NR-heteroaryl, -$NRS(O)_2$ -NR-substituted heteroaryl, -$NRS(O)_2$ -NR-heterocyclic, -$NRS(O)_2$ -NR-substituted heterocyclic, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono-and di-arylamino, mono- and di-(substituted aryl)amino, mono- and di-heteroarylamino, mono- and di-(substituted heteroaryl)amino,

mono- and di-heterocyclic mono- and di-(substituted heterocyclic) amino, unsymmetric di-substituted amines having different substituents selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic, substituted alkynyl groups having amino groups blocked by conventional blocking groups (such as Boc, Cbz, formyl, and the like) and alkynyl/substituted alkynyl groups substituted with $-SO_2$ -alkyl, $-SO_2$ - substituted alkyl, $-SO_2$ -alkenyl, $-SO_2$ -substituted alkenyl, $-SO_2$ -cycloalkyl, $-SO_2$ -substituted cycloalkyl, $-SO_2$ -aryl, $-SO_2$ -substituted aryl, $-SO_2$ -heteroaryl, $-SO_2$ - substituted heteroaryl, $-SO_2$ - heterocyclic, $-SO_2$ -substituted heterocyclic or $-SO_2$ NRR, where R is hydrogen or alkyl.

[0084] Examples of heterocycles and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholino, thiomorpholino, piperidinyl, pyrrolidine, tetrahydrofuranyl, and the like.

[0085] "Heterocyclyloxy" refers to the group -O-heterocyclic, and "substituted heterocyclyloxy" refers to the group -O-substituted heterocyclic.

Methods

[0086] There is evidence that the pathogenesis of several similar multisystem drug hypersensitivity reactions involves MHC-restricted presentation of drug or drug metabolites, with direct binding of these non-peptide antigens to MHC molecules or haptenation to endogenous proteins before T cell activation (Svensson et al., 2000). Skin-infiltrating CD8+ cytotoxic T cells were found to be dominant in the bullous reactions such as SJS/TEN (Hari et al., 2001), whereas CD4+ helper T cells were characteristic of milder cutaneous adverse drug reactions, such as maculopapular rash (Pichler et al., 1997). Since the major histocompatibility complex (MHC) is known to be important in determining T-cell mediated immune responsiveness to the antigens, such as metabolites of drugs, we evaluated whether

the alleles of the major histocompatibility complex were associated with drug-induced SJS/TEN/HSS.

[0087] We performed HLA typing on patients with adverse drug reactions. The results indicate that HLA-B*1502 was present in 42 of 42 (100%) SJS/TEN patients who received carbamazepine (Example 1). The allele was also found in 17 of 53 (32%) SJS/TEN patients who received other drugs (8 phenytoin, 2 allopurinol, 2 amoxicillin, 1 sulfasalazine, 1 ketoprofen, 1 Ibuprofen, and 2 unknown drugs). Particularly, 8 of 17 patients (47.05%) who developed SJS/TEN after taking phenytoin also carried the HLA-B*1502 allele. On the other hand, the allele was only found in 4.1% (3/73) of the carbamazepine-tolerant group, 0% (0/32) of the phenytoin-tolerant group, 6.3% (9/142) of the patients who had milder adverse drug reactions other than SJS, and 5.3% (5/94) of the general population. By using the tolerant group as a control, the odds ratio, sensitivity, specificity, positive predictive value, and negative predictive value for B*1502 associated carbamazepine-induced SJS/TEN, were 1712, 100%, 95.89%, 96.0%, and 100%, respectively. For B*1502 associated phenytoin-induced SJS/TEN, the odds ratio, sensitivity, specificity, positive predictive value, and negative predictive value were 58, 47%, 100%, 100%, and 65.35%, respectively. Accordingly, the presence of this HLA-B allele can be used in the identification of high-risk patients for drug-induced SJS/TEN, particularly carbamazepine- and phenytoin- induced SJS/TEN.

[0088] The mild adverse reactions induced by carbamazepine appear to be associated with another allele, HLA-B*4601. Thus, 10 out of 16 (62.5%) of the patients with these milder reactions to carbamazepine had HLA-B*4601. In contrast, the allele was only found in 26% (19/73) of the carbamazepine-tolerant group. The odds ratio for B*4601 associated carbamazepine-induced milder cutaneous ADRs was 4.73. Consequently, HLA-B*4601 can be used in the risk assessment for mild cutaneous ADR induced by carbamazepine.

[0089] A third HLA-B allele, HLA-B*5801, was found in 17 out of 17 (100%) patients with SJS/TEN or hypersensitivity patients who received allopurinol, but only 18% in the general population. The odds ratio, sensitivity, specificity, positive predictive value, and negative predictive value for B*5801 associated allopurinol-induced SJS/TEN or hypersensitivity were 155, 100%, 82%, 84.7%, and 100%, respectively (Example 2). HLA-B*5801 can thus be used to predict the risk for adverse drug reactions in response to allopurinol.

[0090] Accordingly, the present invention provides a method of assessing the risk of a patient for developing an adverse drug reaction after taking a drug, comprising determining the presence of an HLA-B allele selected from the group consisting of HLA-B*1502, HLA-B*5801 and HLA-B*4601, wherein the presence of the HLA-B allele is indicative of a risk for an adverse drug reaction. In a preferred embodiment, HLA-B*1502 is used to predict the risk for SJS/TEN, particularly carbamazepine-induced SJS/TEN.

[0091] Carbamazepine, also known as Tegretol, Tegol, G-32883, Biston, Calepsin, Carbatrol, Epitol, Finlepsin, Sirtal, Stazepine, Telesmin, or Timonil, is an aromatic anticonvulsant. Other aromatic anticonvulsants, including phenytoin (Dilantin) and phenobarbital, cause similar adverse drug reactions as carbamazepine. Therefore, HLA-B*1502 can be employed to assess the risk for adverse drug reactions to these other aromatic anticonvulsants as well. The aromatic anticonvulsants for which HLA-B*1502 can be used as a risk factor also include metabolites and derivatives of carbamazepine, phenytoin or phenobarbital. Metabolites of these drugs are known in the art (see, *e.g.*, Gennis et al., 1991; Leeder, 1998; Naisbitt et al., 2003), such as carbamazepine-10, 11 epoxide, carbamazepine-10, 11-diol, carbamazepine 2,3-diol, dihydro carbamazepine, carbamazepine catechol and carbamazepine o-quinone, p-hydroxy phenytoin, phenytoin dihydrodiol, phenytoin catechol, phenytoin methylcatechol, and phenytoin o-quinone.

[0092] In another preferred embodiment, HLA-B*5801 is used to predict the risk for allopurinol-induced SJS/TEN, or HSS. Allopurinol is a drug for hyperuricemia and chronic gout. As is with the other drugs, HLA-B*5801 can be used to assess the risk of the metabolites and derivatives of allopurinol as well.

[0093] Other subtypes of the HLA-B15, B58 or B46 locus may also be predispositive for cutaneous adverse drug reactions, particularly when the patient is of a different ethnic origin. Such subtype variation has been observed in the art. For example, ankylosing spondylitis is strongly associated with HLA-B27. Many alleles, or subtypes, have been reported for HLA-B27, such as B*2701-B*2723. These subtypes are distributed in different areas in the world, and many are associated with ankylosing spondylitis (Khan, 2000; Feltkamp et al., 2001). We contemplate that HLA-B15, B58 or B46 are associated with cutaneous ADR as described herein, and other subtypes of HLA-B15, B58 or B46 may also be used for risk assessment instead of HLA-B*1502, 5801 or 4601, *e.g.*, HLA-B*1503 or *1558.

[0094] Furthermore, it should be noted that in addition to the specific HLA alleles *per se*, genetic markers that are linked to each of the specific alleles can be used to predict the corresponding ADR risk as well. This is because genetic markers near the HLA allele of interest tend to co-segregate, or show a linkage disequilibrium, with the allele of interest. Consequently, the presence of these markers (equivalent genetic markers) is indicative of the presence of the allele of interest, which, in turn, is indicative of a risk for ADR. As shown in Example 3, the HLA-B*1502 haplotype includes HLA markers such as DRB1*1202, Cw*0801, Cw*0806, A*1101, and MICA*019. The HLA markers of the HLA-B*5801 haplotype include, for example, A*3303, Cw*0302, DRB1*0301, and MICA*00201.

[0095] The equivalent genetic marker can be any marker, including HLA markers, microsatellites, and single nucleotide polymorphism (SNP) markers. Preferably, the useful genetic markers are about 200 kb from the HLA-B locus or less. More preferably, the markers are about 100 kb, 80 kb, 60 kb, 40 kb, or 20 kb from HLA-B locus or less. Of particular interest are the markers located between DRB1 and the HLA-A region of a specific HLA-B haplotype.

[0096] The HLA alleles can be detected by using any method known in the art. Preferably, genomic DNA is hybridized to a probe that is specific for the allele of interest. The probe may be labeled for direct detection or contacted by a second, detectable molecule that specifically binds to the probe. Alternatively, cDNA, RNA, or protein product of the allele can be detected. For example, serotyping or microcytotoxity methods can be used to determine the protein product of the allele. Similarly, the equivalent genetic markers can be detected by any methods known in the art.

[0097] To further increase the accuracy of risk prediction, the allele of interest and/or its equivalent genetic marker may be determined along with the genetic markers of accessory molecules and co-stimulatory molecules which are involved in the interaction between antigen-presenting cell and T-cell interaction. These genetic markers include microsatellite and single nucleotide polymorphism (SNP) markers. The accessory and co-stimulatory molecules include cell surface molecules (*e.g.*, CD80, CD86, CD28, CD4, CD8, T cell receptor (TCR), ICAM-1, CD11a, CD58, CD2, etc.) and inflammatory or pro-inflammatory cytokines, chemokines (*e.g.*, TNF-α), and mediators (*e.g.*, complements, apoptosis proteins, enzymes, extracellular matrix components, etc.). Also of interest are genetic markers of drug metabolizing enzymes which are involved in the

bioactivation and detoxification of drugs. These genetic markers also include microsatellite and SNP markers. The drug metabolizing enzymes include phase I enzymes (*e.g.*, cytochrome P450 superfamily etc.) and phase II enzymes (*e.g.*, microsomal epoxide hydrolase, arylamine N-acetyltransferase, UDP-glucuronosyl-transferase, etc.).

[0098] The present invention further provides a method for pharmacogenomic profiling. Thus, a panel of genetic factors is determined for a given individual, and each genetic factor is associated with the predisposition for a disease or medical condition, including adverse drug reactions. In the present method, the panel of genetic factors includes at least one allele selected from the group consisting of HLA-B*1502, 5801 and 4601. The panel preferably includes at least two alleles, and most preferably all three alleles, from the group. In addition to HLA-B*1502, 5801 and/or 4601, the panel may include any other known genetic factors, such as thiopurine methyltransferase and the genes for the long-QT syndrome. The genetic markers for accessory molecules, co-stimulatory molecules and/or drug metabolizing enzymes described above can also be included.

[0099] Further provided is a method of screening and/or identifying medicines that can be used to treat drug-induced SJS/TEN, or HSS by using HLA-B*1502, 5801 or 4601 as a target in drug development. For example, cells expressing any of the alleles can be contacted with medicine candidates, and the candidates that bind to the allele are likely to inhibit the expression and/or function of the allele. The efficacy of the candidate in treating drug induced adverse reactions can then be further tested.

Kits

[00100] Another aspect of the present invention provides a kit comprising the means for detecting at least one allele selected from the group consisting of HLA-B*1502, 5801 and 4601. The means is preferably a probe that binds specifically to the allele, and the kit preferably also contains detection reagents for the probe. The probe is preferably an oligonucleotide. The kit may further comprise tools and/or reagents for collecting biological samples from patients, as well as those for preparing genomic DNA, cDNA, RNA or the allele protein from the samples. For example, PCR primers for amplifying the relevant regions of the genomic DNA may be included.

[00101] The kit preferably comprises means for detecting at least two alleles selected from the group consisting of HLA-B*1502, 5801 and 4601. Optionally, the kit may comprise means for detecting other genetic factors as well, particularly those useful in pharmacogenomic profiling. A preferred example is thiopurine methyltransferase.

[00102] Thus, in a preferred embodiment, the kit may comprise probes for detecting all three alleles, HLA-B*1502, 5801 and 4601. More preferably, the kit further comprises the PCR primers suitable for each and every allele as well.

[00103] The following examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of the present invention. While this invention is particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

EXAMPLES

[00104] In the examples below, the following abbreviations have the following meanings. Abbreviations not defined have their generally accepted meanings.

[00105] °C = degree Celsius

[00106] hr = hour

[00107] min = minute

[00108]	sec	=	second
[00109]	μM	=	micromolar
[00110]	mm	=	millimolar
[00111]	M	=	molar
[00112]	ml	=	milliliter
[00113]	μl	=	microliter
[00114]	mg	=	milligram
[00115]	μg	=	microgram
[00116]	ADR	=	adverse drug reaction
[00117]	SJS	=	Stevens-Johnson syndrome
[00118]	TEN	=	toxic epidermal necrolysis
[00119]	HSS	=	hypersensitivity syndrome
[00120]	DRESS	=	drug reaction with eosinophilia and systemic symptoms
[00121]	SSO	=	sequence-specific oligonucleotide
[00122]	PCR	=	polymerase chain reaction
[00123]	HLA	=	human leukocyte antigen
[00124]	STRP	=	short tandem repeat polymorphism

Materials and Methods

Patients and control subjects

[00125] For the studies described in Examples 1 and 2, a total of 112 SJS/TEN patients were recruited either from Chang Gung Memorial Hospital or from several other medical centers throughout Taiwan. Among these patients, 42 had carbamazepine (tegretol)-induced SJS/TEN, and 17 had allopurinol-induced severe ADRs. In addition, 126 patients were also enrolled, who had developed a milder hypersensitivity reaction to various drugs. Drug taking history including dosage and duration, and the phenotypes of adverse drug reactions were recorded. The diagnostic criteria of clinical morphology were defined according to Roujeau (Roujeau JC, 1994). We define SJS as skin detachment of less than 10% of body-surface area, overlap SJS-TEN as skin detachment of 10-30%, and TEN as greater than 30%. SJS, overlap

SJS-TEN and TEN are collectively referred to as SJS/TEN.

[00126] For each patient, the suspected drug was withdrawn and the patient observed for symptoms. Patients who developed a cutaneous adverse drug reaction that did not subside upon withdrawal of the drug were excluded. 73 tegretol-tolerant patients were included as controls. Volunteers from the general population of Taiwanese (n= 94; age range: 20 to 80 years) were also recruited. The study was approved by the institutional review board, and informed consent was obtained.

Genotyping for HLA

[00127] Reagents for reverse lineblot sequence-specific oligonucleotide (SSO) were purchased from DYNAL Biotech Ltd. (Bromborough, UK) and used for HLA genotyping. Briefly, a PCR product was generated using biotinylated primer pairs for the second and the third exons of the HLA class I or class II loci, and then hybridized to a lineblot of SSO of probes immobilized on a nylon membrane. The presence of biotinylated PCR product bound to a specific probe is detected using streptavidin-horseradish peroxidase (HRP) and a chromogenic, soluble substrate to produce a blue "line" at the position of the positive probe. The probe reactivity pattern was interpreted by the genotyping software Dynal RELI™ SSO (DYNAL Biotech Ltd.; Bromborough, UK). Potential ambiguities were further resolved by sequence-based typing and DNA sequencing performed according to the IHWG Technical Manual (International Histocompatibility Working Group).

SNP genotyping

[00128] Genomic DNA was isolated by using PUREGENE DNA purification system (Gentra systems, Minnesota, USA). SNP genotyping was performed by high throughput MALDI-TOF mass spectrometry. Briefly, primers and probes were designed using the SpectroDESIGNER software (Sequenom, San Diego, CA, USA). Multiplex polymerase chain reactions (PCR) were performed, the unincorporated dNTPs were dephosphorylated using the shrimp alkaline phosphatase (Hoffman-LaRoche, Basel, Switzerland), followed by primer extension. The purified primer extension reaction was spotted onto a 384-element silicon chip (SpectroCHIP, Sequenom), analyzed using a Bruker Biflex III MALDI-TOF SpectroREADER mass spectrometer (Sequenom) and spectra processed with SpectroTYPER (Sequenom).

Short tandem repeat polymorphism (STRP) genotyping

[00129] Twenty highly polymorphic microsatellite markers located in the MHC region were selected from NCBI database (*i.e.*, D6S258, D6S2972, D6S510, D6S265, D6S388, D6S2814, HLAC_CA1, HLABC_CA2, MIB, MICA, TNFd, BAT2_CA, D6S273, D6S1615, DQCAR, G51152, D6S2414, D6S1867, D6S1560, and D6S1583). The average heterozygosity of markers was 0.72 with an estimated 230kb of spacing.

[00130] Primers were designed based on oligonucleotide sequence reported within the database. PCR for genotyping was performed in 5-μl volume containing 10 ng of genomic DNA and 0.33 μM each primer by using GeneAmp 9700 thermocyclers (Applied Biosystems, Foster City, CA, USA). Up to 6 products of appropriate size and fluorescent label were pooled before capillary gel electrophoresis. The size of polymorphic amplicons was determined by electrophoresis of ABI 3730 DNA sequencer (Applied Biosystems), using the LIZ500 size standard as an internal size standard (Applied Biosystems). Allele sizing was calculated using the GENMAPPER program version 3.0 (Applied Biosystems). Allele calling and binning were performed using the SAS program. Three CEPH control individuals (1331-01, 1331-02, 1347-2) and H20 were included in all genotyping experiments for quality control purposes.

Statistical analysis

[00131] Allele frequencies in the different groups were compared by the Chi-square method with Yates correction by constructing 2x2 tables. P values were corrected for comparisons of multiple HLA alleles (Pc) by multiplying the raw P values by the observed number of HLA alleles present within the loci. Odds ratios were calculated with Haldane's modification, which adds 0.5 to all cells to accommodate possible zero counts.

EXAMPLE 1

The HLA-B*1502 allele

[00132] In the cohort of 238 individuals with ADRs, 112 cases were diagnosed to have SJS/TEN, and 126 individuals had milder cutaneous adverse drug reactions (erythema multiform, maculopapular rash, urticaria, angioedema, and fixed drug eruption) to various medications. Among the 112 SJS/TEN patients, 42 individuals were exposed to carbamazepine (tegretol), 17 had allopurinol, and 53 were on various medications other than carbamazepine or allopurinol.

[00133] The patients were subject to HLA typing as described in Materials and Methods. As shown in Table 1, a DNA variant allele in the HLA-B locus (HLA-B*1502) was associated in patients with drug-induced SJS/TEN, particularly in patients receiving carbamazepine (tegretol).

Table 1

HLA-B*1502 frequency in 42 Taiwanese patients with carbamazepine (tegretol)-induced Stevens-Johnson syndrome/toxic epidermal necrolysis

Allele	Patients N=42	Controls1[a] N=142	Controls2[b] N=94	Controls3[c] N=73	X^2	Odds Ratio	P_c
B*1502	42 (100%)	9 (6.3)%			137.28	1194.47	3.6×10^{-30}
B*1502	42 (100%)		5 (5.3%)		110.919	1383.2	2.15×10^{-24}
B*1502	42 (100%)			3 (4.1%)	98.936	1712	9.1×10^{-22}

[a], patients who had milder adverse drug reactions other than SJS
[b], general Taiwanese population
[c], patients who are carbamazepine-tolerant
X^2, Chi-square with Yates correction
P_c, calculated by multiplying the raw P values by the observed number of HLA-B alleles (35).

[00134] Thus, HLA-B*1502 was detected in 42 of 42 (100%) SJS/TEN patients who received carbamazepine. The allele was also found in 17 of 53 (32%) SJS/TEN patients who received other drugs (8 phenytoin, 2 allopurinol, 2 amoxicillin, 1 sulfasalazine, 1 ketoprofen, 1 Ibuprofen, and 2 unknown drugs). Particularly, 8 of 17 patients (47.05%) who developed SJS/TEN after taking phenytoin also carried the HLA-B*1502 allele. On the other hand, the

allele was only found in 4.1% (3/73) of the carbamazepine-tolerant group, 0% (0/32) of the phenytoin-tolerant group, 6.3% (9/142) of the patients who had milder adverse drug reactions other than SJS, and 5.3% (5/94) of the general population. By using the tolerant group as a control, the odds ratio, sensitivity, specificity, positive predictive value, and negative predictive value for B*1502 associated carbamazepine-induced SJS/TEN, were 1712, 100%, 95.89%, 96.0%, and 100%, respectively. With such a high predictive value and sensitivity, typing of this HLA-B allele can be used in identifying high-risk patients for drug-induced SJS/TEN, particularly tegretol-induced SJS/TEN.

[00135] The B*1502 allele does not appear to be associated with all phenotypes induced by tegretol. As shown in Table 2, the allele was not detected in the 16 patients suffering from milder cutaneous reactions to tegretol, such as maculopapular rash (Table 2). However, another allele, HLA-B*4601, is significantly associated with these milder cutaneous reactions (10 out of 16 patients, or 62.5%). Therefore, HLA-B*4601 can be used as a risk factor for mild cutaneous ADRs, particularly those induced by tegretol.

Table 2

Phenotype/genotype data of patients with tegretol-induced cutaneous ADRs

ID	Suspected drug	Phenotype	HLA-B Genotype
1	Carbamazepine	SJS	B*1502/B*3802
2	Carbamazepine	SJS	B*1502/B*3501
3	Carbamazepine	SJS	B*1502/B*4006
4	Carbamazepine	SJS	B*1502/B*3802
5	Carbamazepine	SJS	B*1502/B*3802
6	carbamazepine, phenytoin	SJS	B*1502/B*3802
7	Carbamazepine	SJS	B*1502/B*4001
8	Carbamazepine	SJS	B*1502/B*3901
9	Carbamazepine	SJS	B*1502/B*5801
10	Carbamazepine	SJS	B*1502/B*5801
11	Carbamazepine	SJS	B*1502/B*1525
12	Carbamazepine	SJS	B*1502/B*4002
13	Carbamazepine	SJS	B*1502/B*4006

ID	Suspected drug	Phenotype	HLA-B Genotype
14	Carbamazepine	SJS	B*1502/B*5801
15	Carbamazepine	Overlap SJS/TEN	B*1301/B*1502
16	Carbamazepine	Overlap SJS/TEN	B*1502/B*3501
17	Carbamazepine	SJS	B*1502/B*3802
18	Carbamazepine	SJS	B*1502/B*4601
19	Carbamazepine	SJS	B*1301/B*1502
20	Carbamazepine	SJS	B*1502/B*5801
21	Carbamazepine	SJS	B*1502/B*4601
22	Carbamazepine, NSAID	SJS	B*1502
23	Carbamazepine	SJS	B*1502/B*3501
24	Carbamazepine	SJS	B*1502/B*4601
25	Carbamazepine	SJS	B*1502/B*4601
26	Carbamazepine	SJS	B*1502/B*5801
27	Carbamazepine	SJS	B*1501/B*1502
28	Carbamazepine	SJS	B*1502/B*4001
29	Carbamazepine	SJS	B*1502
30	carbamazepine, meloxicam, sulidanc, phenytoin	SJS	B*1502/B*5801
31	Carbamazepine	SJS	B*1502/4601
32	Carbamazepine	SJS	B*1502/5801
33	Carbamazepine	SJS	B*1502/4601
34	Carbamazepine	SJS	B*1502/5502
35	Carbamazepine	SJS	B*1502
36	carbamazepine, phenytoin	SJS	B*1502/4002
37	Carbamazepine	SJS	B*1502/4001
38	Carbamazepine	SJS	B*1502
39	carbamazepine, phenytoin	SJS	B*1502
40	Carbamazepine	Overlap SJS/TEN	B*1502/4001

ID	Suspected drug	Phenotype	HLA-B Genotype
41	Carbamazepine	Overlap SJS/TEN	B*1502/4601
42	Carbamazepine	SJS	B*1502/3802
43	Carbamazepine	maculopapular rash	B*5801/B*4601
44	Carbamazepine	erythema multiform	B*4001/B*4601
45	Carbamazepine	maculopapular rash	B*1301/B*4001
46	Carbamazepine	And angioedema	B*4601/B*5401
47	Carbamazepine	maculopapular rash	B*4001/B*4601
48	Carbamazepine, NSAID	maculopapular rash	B*4001/B*4001
49	Carbamazepine	maculopapular rash	B*1301/B*5502
50	Carbamazepine	lip swelling, oral and genital ulcer	B*4601/B*5801
51	Carbamazepine	Maculopapular	B*4601/B*5801
52	Carbamazepine	And angioedema	B*4001
53	Carbamazepine	maculopapular rash	B*4001/B*5101
54	Carbamazepine	maculopapular rash	B*1301/4001
55	Carbamazepine	maculopapular rash	B*4001/B*4601
56	Carbamazepine	erythema multiform	B*4601/B*5401
57	Carbamazepine	maculopapular rash	B*4601
58	Carbamazepine	erythema multiform	B*4601/5101

EXAMPLE 2

The HLA-B*5801 allele and SJS/TEN

[00136] We have also identified HLA-B*5801 allele as a risk factor for the development of allopurinol-induced SJS/TEN. The HLA-B*5801 allele was found in all 17 (100%) SJS/severe ADR patients on allopurinol (Tables 3 and 4), but only in 18% of the general Taiwanese population (odds ratio 155, sensitivity 100%, specificity 82%, positive predictive value 84.7%, negative predictive value 100%, $Pc=3.7\times10^{-9}$). Accordingly, the HLA-B*5801 allele can be used alone or with other genetic markers for risk assessment for development of SJS in individuals taking allopurinol.

Table 3

HLA-B*5801 frequency in 17 Taiwanese patients with allopurinol-induced severe cutaneous adverse drug reactions

Allele	Patients n=17	Controls1[a] n=142	Controls2[b] n=94	X^2	odds ratio	P_c
B*5801	17 (100%)	26(18.3%)		47.2	153.86	2.1×10^{-10}
B*5801	17 (100%)		17 (18.0%)	41.7	155	3.7×10^{-9}

[a], patients who had adverse drug reactions other than allopurinol-induced cutaneous ADR
[b], general Taiwanese population
X^2, Chi-square with Yates correction
P_c, calculated by multiplying the raw P values by the observed number of HLA-B alleles (35).

Table 4

Phenotype/genotype data of patients with allopurinol-induced cutaneous ADRs

Patient ID	Suspected drug	Phenotype	HLA-B Genotype
59	allopurinol	SJS	B*0705/B*5801
60	allopurinol	SJS	B*4001/B*5801
61	allopurinol	SJS	B*1554/B*5801
62	allopurinol	SJS	B*3901/B*5801
63	allopurinol	SJS	B*5801
64	allopurinol	SJS	B*3901/B*5801
65	allopurinol	SJS	B*3901/B*5801
66	allopurinol	SJS	B*4001/B*5801
67	Allopurinol	SJS	B*1502/B*5801
68	allopurinol	SJS	B*4001/B*5801
69	allopurinol	SJS and vasculitis on leg	B*4601/B*5801
70	allopurinol	SJS, and lichenoid	B*4001/B*5801
71	allopurinol	SJS	B*4002/B*5801
72	allopurinol	SJS	B*4001/B*5801
73	alloprinol	SJS	B*5101/B*5801
74	allopurinol	TEN	B*1301/5801
75	alloprinol	SJS	B*5801

EXAMPLE 3

The HLA-B*5801 allele and allopurinol-induced hypersensitivity syndrome

[00137] In a broader study, it was further discovered that HLA-B*5801 is also linked to allopurinol-induced hypersensitivity syndrome (HSS). 31 patients were studied, including 12 SJS, 3 SJS/TEN, 1 TEN, and 15 HSS patients. The criteria for HSS were cutaneous rash (*e.g.*, diffuse macuopapular, exfoliative dermatitis), plus two of the following symptoms: fever, eosinophilia, atypical circulating lymphocytes, leukocytosis, acute hepatocellular injury, or worsening renal function (Arellano et al., 1993). In all enrolled cases, allopurinol was regarded as the offending drug if the onset of ADRs symptoms occurred within the first

2 months of allopurinol exposure and the ADRs symptoms resolved upon withdrawal of the drug. Patients with any of the following conditions were excluded: absence of symptoms after re-exposure to allopurinol, and patients with milder skin eruption who did not meet the criteria of HSS, SJS or TEN.

[00138] The onset of symptoms for all patients was within the first 2 months of allopurinol exposure and 2 patients had a second attack within 2 days of re-exposure. Twelve patients received multiple drugs in addition to allopurinol, but their medical records revealed no adverse drug reactions when these concomitant medications were taken without allopurinol. All patients had hyperuricemia and/or gouty arthritis, as well as other chronic illnesses, including hypertension (14/31), chronic renal disease (16/31), and diabetes (9/31).

[00139] Ninety-eight gouty arthritis patients who had been on allopurinol for at least 6 months (mean= 38 months, range= 6-107 months) with no evidence of ADRs were included as the allopurinol-tolerant control. The sex distribution of tolerant group is comparable to general prevalence of gout in Chinese people. Furthermore, 93 normal subjects served as an additional control group, as the general population of Taiwan. The demographic variables of these 3 groups are shown in Table 5.

Table 5

Demographic variables, dosage and duration of allopurinol exposure in severe ADRs patients, tolerant patients, as well as normal subjects

	Severe ADRs (n=31)	Tolerant (n=98)	Normal Subjects (n=93)
Sex			
Male	12	89	52
Female	19	9	41
Age (years)			
Median (range)	57.9 (18-91)	57.3 (21-84)	53.9 (22-91)
Allopurinol dosage (mg/day)			
Median (range)	143.3 (50-300)	159.2 (100-400)	None
Duration of allopurinol exposure			
Median (range)	28.2 days (1-56)	38 months (6-107)	None

[00140] HLA-B*5801 allele was present in all 31 (100%) of these 31 allopurinol-induced severe ADRs patients, 16 (16.3%) of the 98 tolerant patients (odds ratio 315, $Pc < 10^{-15}$), and 19 (20%) of the 93 normal subjects (odds ratio 241, $Pc < 10^{-13}$). Relative to the

allopurinol-tolerant group, the absence of this allele had a negative predictive value of 100% for allopurinol-ADRs, and the presence of B*5801 had a positive predictive value of 66%. Therefore, HLA-B*5801 is a marker with high specificity (84%) and sensitivity (100%) for allopurinol-induced severe ADRs, including cutaneous ADRs and allopurinol-induced DRESS (drug reaction with eosinophilia and systemic symptoms).

EXAMPLE 4

Equivalent genetic markers that are linked to the HLA-B*1502 or B*5801 allele

[00141] The presence of HLA-B*1502, 5801 or 4601 may be indicated by other genetic markers. In particular, genetic markers near the HLA allele of interest tend to co-segregate, or show a linkage disequilibrium, with the allele of interest. Consequently, the presence of these markers (equivalent genetic markers) is indicative of the presence of the allele of interest.

[00142] To test the incidence of potential equivalent genetic markers in patients with adverse drug reactions, several markers in the HLA-B*1502 haplotype were determined for their association of adverse drug reactions. Indeed, HLA markers of the HLA-B*1502 haplotype, such as DRB1*1202, Cw*0801, Cw*0806, A*1101, and MICA*019, had a significantly higher incidence in SJS/TEN patients who had been exposed to carbamazepine (Table 6).

Table 6

Contribution of markers of B*1502- ancestral haplotypes to susceptibility to adverse drug reactions

	CBZ SJS/TEN (n=42)	CBZ Milder (n=16)	CBZ Tolerant (n=73)	Allopurinol SJS/TEN (n=17)	General Population (n=94)
HLA-B*1502	42 (100%)	0 (0%)	3 (4.1%)	1 (5.8%)	5 (5.3%)
HLA-Cw*0801	38 (90%)	ND	10 (13.7%)	2 (11.7%)	10 (10.6%)
HLA-Cw*0806	3 (7.1%)	ND	0 (0%)	0 (0%)	0 (0%)
HLA-A*1101	31 (73.8%)	ND	ND	ND	28 (29.8%)
HLA-DRB1*1202	35 (83.3%)	ND	ND	ND	19 (20.2%)

[00143] Markers associated with HLA-B*5801 were also determined. Guided by 4 patients who were homozygous for the HLA-B*5801 alleles, we analyzed the allele distribution of the combined HLA loci and defined the ancestral haplotype as HLA-A*3303, Cw*0302, B*5801 and DRB1*0301. This ancestral haplotype was presented in 12 (38.7%) of the 31 allopurinol-ADRs patients (Table 7), but only in 7.1% of the tolerant patients and 9.7% of the normal subjects.

Table 7

Frequencies of individual or combined loci of HLA-B*5801 ancestral haplotype in patients with allopurinol-induced severe adverse drug reactions, allopurinol tolerant and normal subjects

	Allopurinol-ADRs (n=31)	Allopurinol-tolerant (n=98)	Normal Subjects (n=93)
B*5801	31 (100%)	16 (16.3%)†	19 (20.4%)‡
Cw*0302	29 (93.5%)	15 (15.3%)	19 (20.4%)
A*3303	20 (64.5%)	18 (18.4%)	20 (21.5%)
DRB1*0301	21 (67.7%)	14 (14.3%)	14 (15.1%)
B*5801, Cw*0302	29 (93.5%)	15 (15.3%)	19 (20.4%)
B*5801, Cw*0302, A*3303	20 (64.5%)	13 (13.3%)	16 (17.2%)
B*5801, Cw*0302, DRB1*0301	19 (61.3%)	9 (9.2%)	10 (10.8%)
B*5801, Cw*0302, A*3303, DRB1*0301	12 (38.7%)	7 (7.1%)	9 (9.7%)

† Odds ratio (Allopurinol-ADRs/Tolerant): 315 (95% CI, 18.3-5409.5), $p_c = 7.5 \times 10^{-16}$.

‡ Odds ratio (Allopurinol-ADRs/Normal): 241 (95% CI, 14.1-4111), $p_c = 6.1 \times 10^{-14}$.

[00144] We also independently analyzed haplotypes by typing STRP (short tandem repeat polymorphism) markers in the MHC region. A linkage disequilibrium plot showed a block located between HLA-C and TNFd in the allopurinol-ADRs patient group, but not in the tolerant group. In this block, a haplotype (MIB*358-MICA*206-TNFd*140) near the HLA-B gene was identified, which is consistent with the finding of the association of B*5801 with the disease (p=0.0018). By using STRP markers and sequencing of the MICA allele, we discovered that all allopurinol-ADRs patients analyzed carried the same B allele (B*5801), MICA allele (MICA*00201) and TNF STRP marker (TNFd*140). One patient, however, did not have the same MIB marker (MIB*358) as the rest of patients. These data

indicate that the susceptibility genes lie in the 230 kb region between HLA-B and TNF loci excluding the MIB.

We claim:

1. An *in-vitro* method of assessing the risk of a patient for developing an adverse drug reaction in response to a drug, comprising determining from a biological sample, the presence of an HLA-B allele selected from the group consisting of HLA-B* 1502, HLA-B*5801 and HLA-B*4601, wherein the presence of the HLA-B allele is indicative of a risk for an adverse drug reaction.

2. The method of claim 1 wherein the drug is selected from the group consisting of carbamazepine, allopurinol, phenytoin, sulfasalazine, amoxicillin, ibuporfen and ketoprofen.

3. The method of claim 1 or 2 wherein the drug is carbamazepine.

4. The method of claim 1 or 2 wherein the drug is allopurinol.

5. The method of any of claims 1-4 wherein the adverse drug reaction is Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), or drug hypersensitivity syndrome (HSS).

6. The method of claim 1, 2, 3 or 5 wherein the adverse drug reaction is SJS or TEN, the drug is carbamazepine, and the allele is HLA-B*1502.

7. The method of claim 1, 2, 4 or 5 wherein the adverse drug reaction is SJS, TEN, or HSS, the drug is allopurinol, and the allele is HLA-B*5801.

8. The method of claim 1, 2, or 5 wherein the adverse drug reaction is SJS, TEN, or HSS, the drug is phenytoin, and the allele is HLA-B*1502.

9. The method of any of claims 1-8 wherein the presence of the allele is determined by using an oligonucleotide that specifically hybridizes with the nucleic acid coding for the allele.

10. The method of any of claims 1-9 wherein the presence of the allele is determined by using DNA prepared from the peripheral blood of the patient.

11. The method of any of claims 1-9 wherein the presence of the allele is determined by using RNA, protein, cells or sera prepared from the peripheral blood of the patient.

12. The method of any of claims 1-11 the presence of the allele is determined by assaying for an equivalent genetic marker of the allele, wherein the presence of the equivalent genetic marker is indicative of the presence of the allele.

13. The method of claim 12 wherein the equivalent genetic marker is selected from the group consisting of HLA-DRB1*1202, Cw*0801, Cw*0806, A*1101, MICA*019, A*3303, Cw*0302, DRB1*0301, and MICA*00201.

14. An *in-vitro* method for developing a therapy for an adverse reaction induced by a drug, comprising screening candidate medicines using an *in-vitro* assay in which at least one HLA-B allele is a target, wherein the HLA-B allele is selected from the group consisting of HLA-B*1502, HLA-B*5801, and HLA-B*4601.

15. The method of claim 14 wherein the adverse reaction is Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), or drug hypersensitivity syndrome (HSS).

16. The method of claim 14 or 15 wherein the drug is selected from the group consisting of carbamazepine, allopurinol, phenytoin, sulfasalazine, amoxicillin, ibuporfen and ketoprofen.

17. The method of claim 14, 15 or 16 wherein the drug is carbamazepine or allopurinol.

18. The method of any of claims 14-17 wherein the adverse reaction is SJS or TEN, the drug is carbamazepine, and the allele is HLA-B*1502.

19. The method of any of claims 14-17 wherein the adverse reaction is SJS, TEN, or HSS, the drug is allopurinol, and the allele is HLA-B*5801.

20. The method of any of claims 14-19 wherein the *in-vitro* assay comprises providing a cell that expresses the HLA-B allele.

21. An *in-vitro* method of pharmacogenomics profiling comprising determining from a biological sample, the presence of at least two HLA-B allele selected from the group consisting of HLA-B*1502, HLA-B*5801, and HLA-B*4601.

22. The method of claim 21 wherein the presence of both HLA-B* 1502 and HLA-B*5801 is determined.

23. The method of claim 21 or 22 further comprising determining the presence of at least one genetic factor selected from the group consisting of thiopurine methyltransferase and the genes for the long-QT syndrome.

24. The method of any of claims 21-23 wherein the presence of the allele is determined by using an oligonucleotide that specifically hybridizes with the nucleic acid coding for the allele.

25. The method of any of claims 21-24 wherein the presence of the allele is determined by using DNA prepared from the peripheral blood of the patient.

26. The method of any of claims 21-24 wherein the presence of the allele is determined by using RNA, protein, cells or sera prepared from the peripheral blood of the patient.

27. An *in-vitro* method for identifying a drug that induces SJS, TEN or HSS, comprising contacting, *in-vitro*, a cell that comprises an HLA subtype with a candidate drug, wherein the HLA subtype is an HLA-B*1502, HLA-B*5801 or HLA-B*4601.