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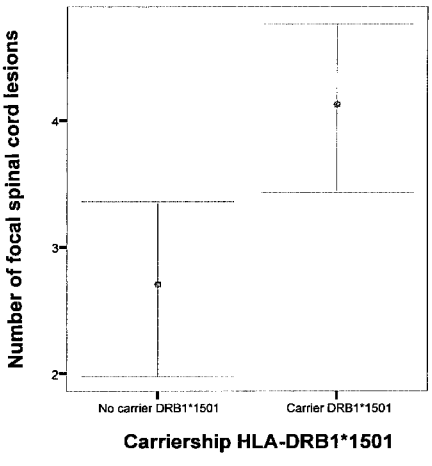
(54) Title: A GENOTYPING TOOL FOR IMPROVING THE PROGNOSTIC AND CLINICAL MANAGEMENT OF MS PATIENTS



(57) Abstract: The invention relates to methods of evaluating MS severity based on analysis of single nucleotide polymorphisms (SNPs) and to products and kits for use in such methods. The methods include a method of assessing a multiple sclerosis disease severity phenotype in a human subject having multiple sclerosis, by determining the genotype of the subject at one or more positions of single nucleotide polymorphism (SNP) selected from: rs2107538, rsl 137933, rsl318, rs2069763, rs423904, rs876493, rsl0243024, rsl0259085, rslO42173, rsl0492503, rsl0492972, rsl2047808, rsl2202350, rsl2861247, rsl3353224, rsl350666, rsl555322, rsl611115, rsl7641078, rsl805009, rs2028455, rs2032893, rs2049306, rs2066713, rs2074897, rs2076530, rs2187668, rs2213584, rs2227139, rs2234978, rs2239802, rs2395182, rs260461, rs28386840, rs3087456, rs3135388, rs3741981, rs375645O, rs3781202, rs3787283, rs38O8585, rs4128767, rs4404254, rs4473631, rs4680534, rs6077690, rs6457594, rs6570426, rs659366, rs6917747, rs7208257, rs7528684, rs7577925, rs762550, rs7956189, rs7995215, rs8049651, rs8702, rs9808753 and rs987107, and/or a SNP in linkage disequilibrium with any one of said SNPs.

WO 2010/103292 A2

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A GENOTYPING TOOL FOR IMPROVING THE PROGNOSTIC AND CLINICAL MANAGEMENT OF MS PATIENTS

FIELD OF THE INVENTION

The invention relates to methods and products, in particular microarrays, for in vitro genotyping of multiple sclerosis (MS) associated genetic variations and to methods for assessment of MS disease severity.

BACKGROUND OF THE INVENTION

Multiple Sclerosis is an autoimmune chronic inflammatory disease, characterized by a progressive demyelination of the central nervous system. While its origin still remains unknown, its multifactorial etiology is well known, consisting of a clear genetic component regulated by several environmental factors.

Clinical evolution of MS is very heterogeneous, and there are different phenotypes present. These range from a very severe form where patients worsen rapidly (known as primary progressive MS), to a more benign form, where the patient practically recovers completely after each disease relapse (known as relapsing remitting MS). Nowadays, disease diagnostics is clinically based, relying on three main points: clinical history, neurologic exploration and use of several techniques (Magnetic Resonance Imaging, analysis of cerebrospinal fluid and evoked potentials).

Currently there is no treatment that will cure MS. MS therapies aim at controlling symptoms and maintaining patient's quality of life. With such treatments, the number of relapses is controlled to a certain level, allowing partial prevention of consequences that may cause such relapses. The primary aims of therapy are returning function after an attack, preventing new attacks, and preventing disability. As with any medical treatment, medications used in the management of MS have several adverse effects. Disease-modifying treatments reduce the progression rate of the disease, but do not stop it. As multiple sclerosis progresses, the symptomatology tends to increase. The disease is associated with a variety of symptoms and functional deficits that result in a range of progressive impairments and disability.

Management of these deficits is therefore very important. Both drug therapy and neurorehabilitation have shown to ease the burden of some symptoms, though neither influences disease progression. As for any patient with neurologic deficits, a multidisciplinary

approach is key to limiting and overcoming disability; however, there are particular difficulties in specifying a 'core team' because people with MS may need help from almost any health profession or service at some point. Similarly, for each symptom there are different treatment options. Treatments should therefore be individualized depending both on the patient and the physician.

SUMMARY OF THE INVENTION

Aspects of the invention relate to methods of analyzing a patient's genotype, for example through analysis of SNPs, optionally combined with clinical-environmental data, for prognosis and treatment management of MS patients, leading to personalized medicine. Accordingly, in a first aspect the present invention provides a method of assessing a MS disease severity phenotype in a human subject having or suspected of having MS, the method comprising determining the genotype of the subject at one or more positions of single nucleotide polymorphism (SNP) selected from those listed in Table 10 and/or a SNP in linkage disequilibrium with any one of said SNPs. The SNPs may be as disclosed in the NCBI dbSNP build 131, *Homo sapiens* genome build 37.1 and/or NCBI dbSNP build 129, *Homo sapiens* build 36.3. The presence of one or more "risk alleles" as identified in Table 10 at one or more of the SNPs indicates that the subject has a higher probability of having a greater severity of MS. In some cases, the method of this and other aspects of the invention comprises determining that the subject does have at least one risk allele at at least one of said SNPs. In other cases, the subject may be determined to be free from said risk alleles at at least one of said SNPs. In some cases, the method of this and other aspects of the invention, the presence of:

the TT genotype at rs2107538;

the GG genotype at rs1137933;

the AA genotype at rs1318;

the GG genotype at rs2069763;

the CC genotype at rs423904;

the AA genotype at rs876493;

the GG genotype at rs10243024;

the GG genotype at rs10259085;

the AA genotype at rs1042173;
the TT genotype at rs10492503;
the GG genotype at rs10492972;
the GG genotype at rs12047808;
the AA genotype at rs12202350;
the GG genotype at rs12861247;
the AA genotype at rs13353224;
the GG genotype at rs1350666;
the AA genotype at rs1555322;
the AA genotype at rs1611115;
the GG genotype at rs17641078;
the GG genotype at rs1805009;
the GG genotype at rs2028455;
the AA genotype at rs2032893;
the AA genotype at rs2049306;
the AA genotype at rs2066713;
the AA genotype at rs2074897;
the GG genotype at rs2076530;
the AA genotype at rs2187668;
the AA genotype at rs2213584;
the CC genotype at rs2227139;
the TT genotype at rs2234978;
the GG genotype at rs2239802;
the GG genotype at rs2395182;
the AA genotype at rs260461;
the AA genotype at rs28386840;
the GG genotype at rs3087456;
the AA genotype at rs3135388;
the AA genotype at rs3741981;
the AA genotype at rs3756450;
the CT genotype at rs3781202;

the AA genotype at rs3787283;
the AA genotype at rs3808585;
the GG genotype at rs4128767;
the GG genotype at rs4404254;
the CC genotype at rs4473631;
the AA genotype at rs4680534;
the TT genotype at rs6077690;
the AA genotype at rs6457594;
the TT genotype at rs6570426;
the CC genotype at rs659366;
the GG genotype at rs6917747;
the AA genotype at rs7208257;
the GG genotype at rs7528684;
the AA genotype at rs7577925;
the AA genotype at rs762550;
the GG genotype at rs7956189;
the GG genotype at rs7995215;
the AA genotype at rs8049651;
the GG genotype at rs8702;
the GG genotype at rs9808753; and/or
the AA genotype at rs987107 is indicative of the subject having, or having a high probability of having, a more severe multiple sclerosis disease phenotype.

In some cases, a more severe multiple sclerosis disease phenotype may be a phenotype selected from: a multiple sclerosis severity score (MSSS) of 2.5 or greater; an increase in size and/or distribution of T2 brain lesions; an increased number of focal lesions in the spinal cord; an increased T2 lesion load in the brain; and the presence of diffuse abnormalities in the spinal cord. Optionally, the method of this and other aspects of the invention may comprise determining the genotype of the subject at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55 or more of said SNPs. Optionally, the method of the invention further comprises the

measurement of at least one clinical variable, such as a clinical variable is selected from: age of the subject at onset of multiple sclerosis, gender of the subject and type of multiple sclerosis at onset of multiple sclerosis. The method of the invention may, in some cases, comprise determining the genotype of the subject at a specific combination or sub-set of SNPs selected from those listed in Table 10, such as the first 2, first 3, first 4, first 5 or first 6. Accordingly, in some cases, the method of the invention comprises determining the genotype of the subject at: at least rs2107538, rs1137933 and rs1318; at least rs2107538, rs1137933, rs1318, rs2069763, rs423904 and rs876493. In some cases, the method of the invention comprises determining the genotype of the subject at substantially all of the SNPs listed in Table 10. In some cases, the method of the invention comprises determining the genotype of the subject at only the SNPs listed in Table 10 and/or only SNPs in linkage disequilibrium with one or more of the SNPs listed in Table 10.

In certain cases, the method of the invention comprises determining the genotype of the subject at a sub-set of SNPs of those listed in Table 10, which sub-set is indicative of a particular MS disease severity phenotype. Methods for assessing particular MS disease severity phenotypes, such as a multiple sclerosis severity score (MSSS) of 2.5 or greater; an increase in size and/or distribution of T2 brain lesions; an increased number of focal lesions in the spinal cord; an increased T2 lesion load in the brain; and the presence of diffuse abnormalities in the spinal cord, may be combined to yield assessment of multiple specific MS disease severity phenotypes or performed independently.

In particular, the method of the invention may be for assessing multiple sclerosis severity score (MSSS), such as whether or not the subject has an MSSS score of 2.5 or greater, wherein the method comprises determining the genotype of the subject at at least 2 of the following positions of SNP: rs423904, rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3756450, rs12047808, rs10259085, rs1042173, rs6077690, rs1611115, rs4473631, rs2032893, rs2066713, rs260461, rs3787283, rs6917747, rs2049306, rs12861247, rs4404254, rs4680534, rs17641078, rs2187668, rs7528684, rs7577925, rs1805009, rs3741981, rs12202350, rs28386840, rs2028455, rs10492503, rs8049651, rs13353224, rs1555322, rs10243024 and rs6570426, wherein the presence of one or more of the risk

alleles shown in Table 10 at one or more of said SNPs is indicative of having an MSSS score of 2.5 or greater. For example, the method may comprise determining the genotype of the subject at at least the following positions of SNP: rs2107538, rs1137933, rs1318, rs2069763, rs423904 and rs876493. Methods for assessing multiple sclerosis severity score (MSSS) of a subject may advantageously combine genotyping SNPs as specified above with determining at least 1, 2 or 3 clinical variables selected from: age of the subject at onset of multiple sclerosis, gender of the subject and type of multiple sclerosis at onset of multiple sclerosis. Thus, the method of the invention may comprise assessment of MSSS score utilising a model which combines the SNPs and clinical variables shown in Table 3, Table 3B and/or Table 3C, optionally employing the respective coefficient for each SNP and/or clinical variable shown in column "B" of said table or tables

In certain cases, the method of this and other aspects of the invention may be for assessing the probability of increased size and/or distribution of T2 brain lesions in the subject, wherein the method comprises determining the genotype of the subject at at least 2, 3 or 4 of the following positions of SNP: rs2213584, rs2227139, rs2076530 rs876493, rs9808753, rs2074897, rs762550, rs2234978, rs3781202.

In certain cases, the method of this and other aspects of the invention may be for assessing the probability of increased T2 lesion load in the brain, wherein the method comprises determining the genotype of the subject at at least 1, 2, 3 or 4 of the following positions of SNP: rs2107538, rs12861247, rs2074897 and rs7995215, such as determining the genotype of the subject at: rs12861247, rs2074897 and rs7995215.

In certain cases, the method of this and other aspects of the invention may be for assessing an increased number of focal lesions in the spinal cord, wherein the method comprises determining the genotype of the subject at at least 1, 2, 3 or 4 of the following positions of SNP: rs3135388, rs2395182, rs2239802, rs2227139, rs2213584, rs3087456, rs10492972, rs12202350, rs8049651, rs8702 and rs987107, such as determining the genotype of the subject at: rs3135388, rs3087456 and rs2227139.

In certain cases, the method of this and other aspects of the invention may be for assessing the presence of diffuse abnormalities in the spinal cord, wherein the method comprises determining the genotype of the subject at at least 1, 2, 3 or 4 of the following positions of SNP: rs1350666, rs3808585, rs4128767, rs6457594, rs7208257 and rs7956189.

The method in accordance with this and other aspects of the invention may, in some cases, be carried out *in vitro* using a nucleic acid-containing sample that has been obtained from the subject. In some cases the genotype of the subject at said one or more positions of SNP may be determined indirectly by determining the genotype of the subject at a position of SNP that is in linkage disequilibrium with said one or more positions of SNP, while in some cases the genotype of the subject at said one or more positions of SNP may be determined directly by identifying one or both alleles at said one or more positions of SNP.

In accordance with the method of this and other aspects of the invention, determining the genotype of the subject at said one or more positions of SNP may comprise:

(i) extracting and/or amplifying DNA from a sample that has been obtained from the subject;

(ii) contacting the DNA with an array comprising a plurality of probes suitable for determining the identity of at least one allele at a position of SNP as listed in Table 10, for example using one or more probes selected from those listed in Table 7. In some cases, the array may be a DNA array, a DNA microarray or a bead array.

In accordance with the method of this and other aspects of the invention the method may comprise amplifying DNA from a sample that has been obtained from the subject, wherein said amplifying comprises contacting the DNA with at least one forward primer as listed in Table 8 and at least one reverse primer as listed in Table 9.

In a further aspect, the present invention provides an array of probes for use in a method according to the invention, wherein the array comprises:

at least 5, 10, 15, 20, 50 or more nucleic acid probes suitable for determining the identity of at least one allele at a position of SNP as listed in Table 10; and

a solid support on which said probes are immobilised,

wherein said probes comprise at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% of the total number of nucleic acid probes in the array, or essentially all of the nucleic acid probes in the array. The probes suitable for determining the identity of at least one allele at a position of SNP may be selected from the probes listed in Table 7.

In a further aspect, the present invention provides methods of evaluating disease severity in a patient having multiple sclerosis, including obtaining a DNA sample from the patient, and determining the presence or absence of two or more single nucleotide polymorphisms (SNPs) associated with severity of the disease, wherein the presence of two or more SNPs associated with severity of the disease indicates a likelihood of increased disease severity. In some embodiments the two or more SNPs associated with the disease comprise SNPs in PNMT, IL1R, CCL5, IL2, PITPNC1 or NOS2A. In certain embodiments the two or more SNPs are selected from those listed in Table 10. In certain embodiments, the two or more SNPs associated with the disease are selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763 and rs2107538. In certain embodiments the two or more SNPs associated with the disease are selected from the group consisting of rs3135388, rs2395182, rs2239802, rs2227139, rs2213584, rs3087456 and rs2107538.

The presence or absence of two or more SNPs associated with severity of the disease can be determined by any method known in the art such as a gene chip, bead array, RFLP analysis, and/or sequencing. In some embodiments the two or more SNPs associated with the disease comprise SNPs in PNMT, IL1R, CCL5, IL2, PITPNC1 or NOS2A. In certain embodiments the two or more SNPs associated with the disease are selected from the group consisting of, rs876493, rs1137933, rs1318, rs2069763 and rs2107538. In certain embodiments the two or more SNPs associated with the disease are selected from the group consisting of rs1137933, rs1318, rs2069764 and rs2107538.

Aspects of the invention relate to SNPs associated with increased T2 lesion load in the brain. In some embodiments the SNP is associated with an increased number of focal spinal cord abnormalities. In some embodiments the two or more SNPs are in linkage

disequilibrium. In certain embodiments the two or more SNPs are in linkage disequilibrium with SNPs selected from the group consisting of rs2239802, rs2213584, rs3135388, 2213584 rs2227139, rs1137933, rs1318, rs2069764 and rs2107538.

In some embodiments methods described herein further include the measurement of one or more clinical variables such as age of onset, gender, and/or type of onset of disease. In some embodiments disease severity is based on an MS severity scale such as the Multiple Sclerosis Severity Score (MSSS) test, the Kurtzke Expanded Disability Status Scale (EDSS), or the Multiple Sclerosis Functional Composite (MSFC) measure.

In some embodiments the presence or absence of at least 6 SNPs is determined. In certain embodiments the two or more SNPs are selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456. In certain embodiments at least one of the SNPs is in linkage disequilibrium with a SNP selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456. In some embodiments methods described herein include use of one or more probe sets listed in Table 7. In some embodiments methods described herein include at least one forward primer from Table 8 and one reverse primer from Table 9.

Aspects of the invention relate to methods of designing a treatment regimen for a patient having multiple sclerosis, including obtaining a DNA sample from the patient, determining the presence or absence of two or more single nucleotide polymorphisms (SNPs) associated with severity of the disease, wherein the presence of two or more SNPs associated with severity of the disease indicates a likelihood of increased disease severity, and designing the treatment regimen based on the presence or absence of the SNPs associated with the disease. In some embodiments the treatment regimen comprises early or elevated doses of glatiramer acetate, vitamin D, interferon beta-1a or -1b, natalizumab, mitoxantrone, and/or corticosteroids.

Aspects of the invention relate to methods of treating a patient having a prognosis of increased disease severity, comprising early or elevated doses of glatiramer acetate, vitamin D, interferon beta-1a or -1b, natalizumab, mitoxantrone, and/or corticosteroids.

Aspects of the invention relate to methods of identifying SNPs associated with severity of symptoms in multiple sclerosis, including obtaining a DNA sample from a patient having multiple sclerosis, identifying SNPs in the DNA, wherein the SNPs comprise two or more of the SNPs listed in Table 1, performing an MRI on the patient to determine spatial distribution of T2 brain lesions, T2 lesion load, presence of diffuse abnormalities and/or number of spinal cord lesions, comparing identified SNPs with the spatial distribution of T2 brain lesions, T2 lesion load, presence of diffuse abnormalities and/or number of spinal cord lesions, and identifying the SNPs that correlate with spatial distribution of T2 brain lesion, T2 lesion load, presence of diffuse abnormalities and/or number of spinal cord lesions, wherein the SNPs that correlate with spatial distribution of T2 brain lesions, T2 lesion load, presence of diffuse abnormalities and/or number of spinal cord lesions, are SNPs associated with severity of symptoms in multiple sclerosis. In some embodiments at least one of the SNPs is in linkage disequilibrium with a SNP listed in Table 1. In some embodiments identifying SNPs associated with severity of symptoms in multiple sclerosis further comprises consideration of clinical data.

Aspects of the invention relate to methods of evaluating disease severity, as measured using the Multiple Sclerosis Severity Score (MSSS) test, the Kurtzke Expanded Disability Status Scale (EDSS), and/or the Multiple Sclerosis Functional Composite measure (MSFC), in a patient having multiple sclerosis, the method including obtaining a DNA sample from the patient, and determining the presence or absence of two or more single nucleotide polymorphisms (SNPs), wherein said SNPs comprise two or more of the SNPs listed in Table 1, and wherein the presence of said two or more SNPs indicates a likelihood of increased disease severity. In some embodiments evaluating disease severity further comprises consideration of clinical data. In some embodiments at least one of the SNPs is in linkage disequilibrium with a SNP listed in Table 1.

Aspects of the invention relate to methods of evaluating the severity of spinal cord lesions in a patient having multiple sclerosis, the method including obtaining a DNA sample from the patient, and determining the presence or absence of two or more single nucleotide

polymorphisms (SNPs) associated with spinal cord lesions, wherein the presence of two or more SNPs associated with spinal cord lesions indicates a likelihood of increased disease severity. In some embodiments the two or more SNPs are selected from the group consisting of rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456. In certain embodiments one of the SNPs is rs3135388. In some embodiments the two or more SNPs are selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456. In certain embodiments at least one of the SNPs is in linkage disequilibrium with a SNP selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456.

Aspects of the invention relate to method of prognosing the likelihood of T2 lesions and/or T2 lesion load in a patient having multiple sclerosis, the method including obtaining a DNA sample from the patient, and determining the presence or absence of SNP rs2107538, wherein the presence of SNP rs2107538 indicates a likelihood of T2 lesions and/or T2 lesion load in the patient.

Aspects of the invention relate to methods where determining the presence or absence of SNPs includes (a) providing, for each genetic variation to be genotyped, at least 2 oligonucleotide probe pairs, wherein: (i) one pair consists of probes 1 and 2, and the other pair consists of probes 3 and 4; (ii) one probe in each pair is capable of hybridising to genetic variation A and the other probe in each pair is capable of hybridising to genetic variation B; (iii) each probe is provided in replicates; and (iv) the probe replicates are each coupled to a solid support; (c) amplifying and detectably labelling the target DNA; (d) contacting the target DNA with the probes under conditions which allow hybridisation to occur, thereby forming detectably labeled nucleic acid-probe hybridisation complexes, (e) determining the intensity of detectable label at each probe replica position, thereby obtaining a raw intensity value; (f) optionally amending the raw intensity value to take account of background noise, thereby obtaining a clean intensity value for each replica; and (g) applying a suitable algorithm to the intensity data from (e) or (f), thereby determining the genotype with respect to each genetic variation, wherein application of the algorithm comprises calculating an average intensity value from the intensity values for each of the

replicas of each probe and wherein the algorithm uses three Fisher linear functions that characterize each of the three possible genotypes AA, AB or BB for the genetic variation.

Aspects of the invention relate to kits for evaluating severity of disease in a subject having multiple sclerosis, the kit including: (i) at least one set of probes listed in table 7; optionally (ii) instruction for genotyping analysis as described in claim H1; and optionally (iii) instructions for determining the severity MS phenotype from the outcomes. Aspects of the invention relate to PCR amplification kits comprising at least one pair of PCR primers from tables 8 and 9, a thermostable polymerase, dNTPs, a suitable buffer, and optionally instructions for use.

Further aspects of the invention relate to a computational method of deriving a probability function for use in determining an MS severity phenotype in a subject, including applying a probability function such as stepwise multiple logistic regression analysis to outcome data and phenotype data obtained from a suitable study population of individuals, wherein each individual is of known clinically determined phenotype with respect to the Multiple Sclerosis severity phenotype, thereby deriving a probability function which produces a statistically significant separation between individuals of different phenotype in the population; wherein: (i) the phenotype data comprises the known clinically determined phenotype of each individual; (ii) the outcomes data for each individual comprises the genotype of the individual at each SNP in a set of SNPs; and wherein: (a) the probability function is for distinguishing or differentially diagnosing MS severity phenotype, and the set of SNPs is selected from the set of MS severity phenotype discriminating SNPs in Table 3; (b) the probability function is for prognosing MS disease severity phenotype and the set of SNPs is selected from the set of MS disease severity discriminating SNPs in Table 3; and/or (c) the probability function is for prognosing MS disease severity phenotype and the set of SNPs is selected from the set of MS disease severity discriminating SNPs in Table 10.

The present invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or is stated to be expressly avoided. These and further aspects and embodiments of the invention are described in further detail below and with reference to the accompanying examples and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing a ROC (receiver operating characteristic) curve obtained for the model MSSS<2.5 versus ≥2.5, showing the relationship between sensitivity (y-axis) and percentage (x-axis).

Figure 2 depicts MRI data maps showing mean lesion frequency map of the patient sample (n=208). Lesion frequency across the patient sample is shown for every voxel on axial and sagittal slices. The colour bar indicates lesion frequency; voxels with a lesion frequency <1% are not shown; peak frequency was 32%.

Figure 3 depicts MRI data maps showing clusterwise (t=2) associations of lesion presence with genotype, on a background of the common brain image. The cluster colour bar indicates clusterwise p-value, with the range indicated by the colour bar; only clusters with p<0.05 are shown. A: rs2213584 (HLA-DRA gene); B: rs2227139 (HLA- DRA gene); C: rs2076530 (BTNL2 gene); D: rs876493 (PNMT gene).

Figure 4 is a graph showing the mean number of focal spinal cord lesions in patients who carry HLA-DRB1*1501 (measured as presence of A-allele of rs3135388). Difference between carriers and non-carriers p<0.001, Mann Whitney U test. Error bars show 95% confidence interval of mean.

Figure 5 is a graph showing a ROC (receiver operating characteristic) curve obtained for the model MSSS<2.5 versus ≥2.5, showing the relationship between sensitivity (y-axis) and percentage (x-axis), as further described in Table 3B.

Figure 6 is a graph showing a ROC (receiver operating characteristic) curve obtained for the model MSSS<2.5 versus ≥2.5, showing the relationship between sensitivity (y-axis) and percentage (x-axis), as further described in Table 3B.

Figure 7 is a graph showing a ROC (receiver operating characteristic) curve obtained for the model MSSS<2.5 versus ≥2.5, showing the relationship between sensitivity (y-axis) and percentage (x-axis), as further described in Table 3C.

DETAILED DESCRIPTION

Multiple Sclerosis (MS) is a multifocal inflammatory demyelinating disease of the central nervous system (CNS), characterized by inflammation, demyelination and axonal loss

resulting in a highly variable clinical presentation. Most patients suffer from relapsing-remitting (RR) MS, experiencing waves of inflammation leading to alternating periods of disability (relapses) and stable disease (remissions). The RRMS phase usually leads to progressive and irreversible disability (the secondary progressive [SP] phase). For a subset of patients, the disease is progressive from onset (primary progressive [PP] MS). Treatment decisions are based on the occurrence of relapses, and the development of white matter lesions visible on MRI. Brain lesion volume and distribution however are highly variable among MS patients, and correlate only moderately with disability. As treatment guidelines would strongly benefit from a better understanding of this variability, the present invention is drawn to methods of genetic screening and predicting severity of disease using genetic information that correlates with increased numbers of lesions in the brain, optic nerve, or spinal cord.

Aspects of the invention relate at least in part to the surprising discovery that MS severity can be associated (e.g., statistically) with one or more genetic markers. As used herein, a genetic marker refers to a DNA sequence that has a known location on a chromosome. Several non-limiting examples of classes of genetic markers include RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), RAPD (random amplification of polymorphic DNA), VNTR (variable number tandem repeat), microsatellite polymorphism, SNP (single nucleotide polymorphism), STR (short tandem repeat), and SFP (single feature polymorphism).

In some embodiments, genetic markers associated with the invention are SNPs. As used herein a SNP or "single nucleotide polymorphism" refers to a specific site in the genome where there is a difference in DNA base between individuals. In some embodiments the SNP is located in a coding region of a gene. In other embodiments the SNP is located in a noncoding region of a gene. In still other embodiments the SNP is located in an intergenic region. It should be appreciated that SNPs exhibit variability in different populations. In some embodiments, a SNP associated with the invention may occur at higher frequencies in some ethnic populations than in others. In some embodiments, SNPs associated with the invention are SNPs that are linked to MS. In certain embodiments a SNP associated with the invention is a SNP associated with a gene that is linked to MS. A SNP that is linked to MS may be identified experimentally. In other embodiments a SNP that is linked to MS may be identified through accessing a database containing information regarding SNPs. Several non-

limiting examples of databases from which information on SNPs or genes that are associated with human disease can be retrieved include: NCBI resources, The SNP Consortium LTD, NCBI dbSNP database, International HapMap Project, 1000 Genomes Project, Glovar Variation Browser, SNPStats, PharmGKB, GEN-SniP, and SNPedia. In some embodiments, SNPs associated with the invention comprise two or more of the SNPs listed in Table 1 and/or Table 10. In some embodiments 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more than 30 SNPs are evaluated in a patient sample. In some embodiments, multiple SNPs are evaluated simultaneously while in other embodiments SNPS are evaluated separately.

SNPs are identified herein using the rs identifier numbers in accordance with the NCBI dbSNP database, which is publically available at: http://www.ncbi.nlm.nih.gov/projects/SNP/ . As used herein, rs numbers refer to the dbSNP build 129, *Homo sapiens* build 36.3 available from 14 April 2008 and/or dbSNP build 131, *Homo sapiens* build 37.1 available from 2 February 2010. Except where indicated otherwise, the rs identifiers are identical for dbSNP build 129, *Homo sapiens* build 36.3 and dbSNP build 131, *Homo sapiens* build 37.1.

In some embodiments, SNPs in linkage disequilibrium with the SNPs associated with the invention are useful for obtaining similar results. As used herein, linkage disequilibrium refers to the non-random association of SNPs at two or more loci. Techniques for the measurement of linkage disequilibrium are known in the art. As two SNPs are in linkage disequilibrium if they are inherited together, the information they provide is correlated to a certain extent. SNPs in linkage disequilibrium with the SNPs included in the models can be obtained from databases such as HapMap or other related databases, from experimental setups run in laboratories or from computer-aided in-silico experiments. Determining the genotype of a subject at a position of SNP as specified herein, e.g. as specified by NCBI dbSNP rs identifier, may comprise directly genotyping, e.g. by determining the identity of the nucleotide of each allele at the locus of SNP, and/or indirectly genotyping, e.g. by determining the identity of each allele at one or more loci that are in linkage disequilibrium with the SNP in question and which allow one to infer the identity of each allele at the locus of SNP in question with a substantial degree of confidence. In some cases, indirect genotyping may comprise determining the identity of each allele at one or more loci that are in sufficiently

high linkage disequilibrium with the SNP in question so as to allow one to infer the identity of each allele at the locus of SNP in question with a probability of at least 90%, at least 95% or at least 99% certainty.

As used herein MS or multiple sclerosis refers to a progressive neurodegenerative disease involving demyelination of nerve cells. Several non-limiting classifications of MS include: relapsing-remitting (RRMS) (typically characterized by partial or total recovery after attacks (also called exacerbations, relapses, or flares)), secondary progressive MS (SPMS) (generally characterized by fewer relapses, with an increase in disability and symptoms), and primary progressive MS (PPMS) (generally characterized by progression of symptoms and disability without remission).

Some non-limiting examples of symptoms of MS include: fatigue (also referred to as MS lassitude), muscle fatigue, paresthesias, difficulty in walking and/or balance problems, abnormal sensations such as numbness, prickling, or "pins and needles", pain, bladder dysfunction, bowel dysfunction, changes in cognitive function (including problems with memory, attention, concentration, judgment, and problem-solving), dizziness and vertigo, emotional problems (e.g., depression), sexual dysfunction, and vision problems. In some embodiments, symptoms of MS can include partial or complete paralysis (such as blurred or double vision, red-green color distortion, or blindness in one eye), headache, hearing loss, itching, seizures, spasticity, speech and swallowing disorders, and tremors. In some embodiments, clinical symptoms of MS can include increased CD4:CD8 cell ratio compared to normal, decreased number of CD14+ cells compared to normal, increased expression of HLA-DR on CD14+ cells compared to normal CD14+ cells, increased levels of activated monocytes or macrophages compared to normal, the presence of proliferating macrophages, and decreased serum IgG and/or IgM compared to normal, where "normal" as used in this context refers to a subject who does not have MS.

Previous studies have explored patterns of spatial lesion distribution in MS patients. Without wishing to be bound by any theory, one potential factor underlying differences in lesion burden and spatial lesion distribution among MS patients may be found in pathological and immunological heterogeneity: studies on spatial lesion distribution throughout the brain demonstrated differences in lesion distribution across disease types and across lesion types. These findings of distinct lesion distributions across patient subgroups and lesion types

suggest that different subtypes of pathology exist in MS based at least in part on different immunological mechanisms. For example, periventricular predilection of MS lesions may be caused at least in part by differences in the vasculature compared to other regions, making this location vulnerable to pathological changes. Without wishing to be bound by any theory, enhanced lesions in peripheral as opposed to central brain regions may be caused, at least in part, by central lesions developing from progressive gliosis and peripheral lesions being more inflammatory. As lesions in different locations may have different immunological backgrounds, they may warrant different treatment mechanisms. Results described herein suggest that differences in immunological backgrounds of lesion formation among MS patients may be driven by genetic predisposition.

Aspects of the invention relate to a large-scale study investigating the genetic influences on different phenotypes of MS (disease severity, subtype, MRI characteristics, response to treatment). Described herein is an investigation into the correlation between genetic background and spatial lesion distribution in a large cohort of MS patients using a variety of SNPs.

Aspects of the invention relate to evaluating the severity of MS in a patient. One symptom associated with MS is the presence of demyelination (lesions or plaques) in the brain and/or spinal cord of a patient. It should be appreciated that regions of demyelination may be detected through any means known to one of ordinary skill in the art. In some embodiments, lesions are detected through MRI. In some embodiments treatment decisions regarding a patient with MS, are based on the occurrence of relapses and the development of white matter lesions visible on MRI. Brain lesion volume and distribution, however, are highly variable among MS patients and correlate only moderately with disability. Treatment guidelines would benefit from a better understanding of this variability. Differences in genetic background may lead to different lesion distribution, which in turn may lead to a different clinical expression of the disease. Thus, the correlations revealed herein, between the presence of specific genetic markers and the presence of lesions offer important applications for screening of patients who have or are at risk of MS, diagnostic and prognostics for MS patients, as well as development of appropriate therapeutic approaches. As used herein, the term disease severity refers to the evaluation of a patient's disability using the tests listed above or other similar tests known in the art. An assessment of disease

severity in some embodiments includes determining rapidity of development of disability, disease duration, rate of progression or relapse of symptoms, and symptoms such as changes in sensation, fatigue, pain, muscle weakness and/or spasm, problems in speech, visual problems, difficulty in moving, difficulties with coordination and balance, bladder and bowel difficulties and cognitive impairment.

Several methods have been established for assessing the severity of MS based on analysis of clinical factors such as those in Table 2, below. Non-limiting examples of tests used to assess the severity of MS include the Kurtzke Expanded Disability Status Scale (EDSS), the Multiple Sclerosis Functional Composite measure (MSFC), and the Multiple Sclerosis Severity Score (MSSS). The MSSS test relates scores on the Expanded Disability Status Scale (EDSS) to the distribution of disability in patients with comparable disease durations. Effectively the MSSS assigns to each EDSS its median decile score within this distribution. For example, an MSSS of 5.0 indicates the disease is progressing at the median rate. A patient whose MSSS is 9.0 is a fast progressor, progressing faster than 90% of patients. A patient whose MSSS is 1.0 is a slow progressor, progressing faster than just 10% of patients. In some embodiments, based on the MSSS test a patient may be assigned a median docile score of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 9.5, including any intermediate values. In some embodiments, based on a test such as the MSSS test, MS patients are allocated into severe and benign subgroups. In some embodiments, MS patients are classified into different categories of severity based on a test such as MSSS. In some embodiments MS patient may be classified as relapsing-remitting (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS).

The invention in one aspect presents a model for assessing the strength of the disability, or the severity of the form of MS, according to the MSSS scale, using SNP analysis, thus allowing differential treatment management for a given patient. Results described herein generate a model from the analysis of 605 MS patients and 700 MS patients (see Example section). In some aspects, the invention evaluates differences between patients that have an MSSS score of less than 2.5 versus patients that have an MSSS score 2.5 or greater. Aspects of the invention relate to using genetic markers that are correlated to certain degrees of MS severity as predictive of MS severity and as indicators of recommended therapeutic approaches. In some embodiments, methods described herein relate to screening a

patient for one or more risk factors associated with MS. In some embodiments the presence of two or more of the SNPs described herein indicate a more severe form of MS.

The invention in one aspect relates to correlating specific SNPs or combinations of SNPs with the presence and/or severity of lesions in the brain and/or spinal cord. The SNPs or combinations of SNPs that are correlated to the presence and/or severity of lesions in the brain and/or spinal cord can be used as predictive, diagnostic or prognostic indicators of the presence and/or severity of lesions in the brain and/or spinal cord. The detection of such SNPs, indicating the presence of lesions in the brain and/or spinal cord may in some embodiments be used as an indicator of the severity of MS.

Aspects of the invention relate to determining the presence of SNPs through obtaining a patient DNA sample and evaluating the patient sample for the presence of two or more SNPs. It should be appreciated that a patient DNA sample can be extracted, and a SNP can be detected in the sample, through any means known to one of ordinary skill in art. Some non-limiting examples of known techniques include detection via restriction fragment length polymorphism (RFLP) analysis, planar microarrays, bead arrays, sequencing, single strand conformation polymorphism analysis (SSCP), chemical cleavage of mismatch (CCM), and denaturing high performance liquid chromatography (DHPLC).

In some embodiments, a SNP is detected through PCR amplification and sequencing of the DNA region comprising the SNP. In some embodiments SNPs are detected using microarrays. Microarrays for detection of genetic polymorphisms, changes or mutations (in general, genetic variations) such as a SNP in a DNA sequence, comprise a solid surface, typically glass, on which a high number of genetic sequences are deposited (the probes), complementary to the genetic variations to be studied. Using standard robotic printers to apply probes to the array a high density of individual probe features can be obtained, for example probe densities of 600 features per cm^2 or more can be typically achieved. The positioning of probes on an array is precisely controlled by the printing device (robot, inkjet printer, photolithographic mask etc) and probes are aligned in a grid. The organisation of probes on the array facilitates the subsequent identification of specific probe-target interactions. Additionally it is common, but not necessary, to divide the array features into smaller sectors, also grid-shaped, that are subsequently referred to as sub-arrays. Sub-arrays

typically comprise 32 individual probe features although lower (e.g. 16) or higher (e.g. 64 or more) features can comprise each subarray.

In some embodiments, detection of genetic variation such as the presence of a SNP involves hybridization to sequences which specifically recognize the normal and the mutant allele in a fragment of DNA derived from a test sample. Typically, the fragment has been amplified, e.g. by using the polymerase chain reaction (PCR), and labelled e.g. with a fluorescent molecule. A laser can be used to detect bound labelled fragments on the chip and thus an individual who is homozygous for the normal allele can be specifically distinguished from heterozygous individuals (in the case of autosomal dominant conditions then these individuals are referred to as carriers) or those who are homozygous for the mutant allele. In some embodiments, the amplification reaction and/or extension reaction is carried out on the microarray or bead itself.

In some embodiments, methods described herein may involve hybridization. For differential hybridization based methods there are a number of methods for analysing hybridization data for genotyping:

Increase in hybridization level: The hybridization levels of probes complementary to the normal and mutant alleles are compared.

Decrease in hybridization level: Differences in the sequence between a control sample and a test sample can be identified by a decrease in the hybridization level of the totally complementary oligonucleotides with a reference sequence. A loss approximating 100% is produced in mutant homozygous individuals while there is only an approximately 50% loss in heterozygotes. In Microarrays for examining all the bases of a sequence of "n" nucleotides ("oligonucleotide") of length in both strands, a minimum of "2n" oligonucleotides that overlap with the previous oligonucleotide in all the sequence except in the nucleotide are necessary. Typically the size of the oligonucleotides is about 25 nucleotides. However it should be appreciated that the oligonucleotide can be any length that is appropriate as would be understood by one of ordinary skill in the art. The increased number of oligonucleotides used to reconstruct the sequence reduces errors derived from fluctuation of the hybridization level. However, the exact change in sequence cannot be identified with this method; in some embodiments this method is combined with sequencing to identify the mutation.

Where amplification or extension is carried out on the microarray or bead itself, three methods are presented by way of example:

In the Minisequencing strategy, a mutation specific primer is fixed on the slide and after an extension reaction with fluorescent dideoxynucleotides, the image of the Microarray is captured with a scanner.

In the Primer extension strategy, two oligonucleotides are designed for detection of the wild type and mutant sequences respectively. The extension reaction is subsequently carried out with one fluorescently labelled nucleotide and the remaining nucleotides unlabelled. In either case the starting material can be either an RNA sample or a DNA product amplified by PCR.

In the Tag arrays strategy, an extension reaction is carried out in solution with specific primers, which carry a determined 5' sequence or "tag". The use of Microarrays with oligonucleotides complementary to these sequences or "tags" allows the capture of the resultant products of the extension. Examples of this include the high density Microarray "Flex-flex" (Affymetrix).

For cost-effective genetic diagnosis, in some embodiments, the need for amplification and purification reactions presents disadvantages for the on-chip or on-bead extension/amplification methods compared to the differential hybridization based methods. However the techniques may still be used to detect and diagnose conditions according to the invention.

Typically, Microarray or bead analysis is carried out using differential hybridization techniques. However, differential hybridization does not produce as high specificity or sensitivity as methods associated with amplification on glass slides. For this reason the development of mathematical algorithms, which increase specificity and sensitivity of the hybridization methodology, are needed (Cutler DJ, Zwick ME, Carrasquillo MN, Yohn CT, Tobi KP, Kashuk C, Mathews DJ, Shah N, Eichler EE, Warrington JA, Chakravarti A. Genome Research; 11 :1913-1925 (2001). Methods of genotyping using microarrays and beads are known in the art.

Some non-limiting examples of genotyping and data analysis can be found in co-pending patent application U.S. Serial No. 11/813,646 (WO 2006/075254), which is hereby incorporated by reference. In some embodiments the genotypes are determined as follows:

The signal from the probes which detect the different genetic variations is determined with a scanner. The scanner software executes a function to subtract the local background noise from the absolute signal intensity value obtained for each probe. Next, the replicates for each of the 4 probes that are used to characterize each genetic variation are grouped. The average intensity value for each of 4 probes is calculated using the average collated from the replicates in order to identify abnormal values (outliers) that can be excluded from further consideration. Once the average intensity value for each of the probes is known then two ratios are calculated (ratio 1 and ratio 2):

$$\text{Ratio 1} = \frac{\text{Average intensity for probe 1}}{\text{Average intensity for probe 1} + \text{Average intensity for probe 2}}$$

$$\text{Ratio 2} = \frac{\text{Average intensity for probe 3}}{\text{Average intensity for probe 3} + \text{Average intensity for probe 4}}$$

wherein probe 1 detects (is capable of specifically hybridising to) genetic variation A (e.g. a normal allele), probe 2 detects (is capable of specifically hybridising to) genetic variation B (e.g. a mutant allele), probe 3 detects (is capable of specifically hybridising to) genetic variation A (e.g. a normal allele) and probe 4 detects (is capable of specifically hybridising to) genetic variation B (e.g. a mutant allele).

These ratios are substituted in three Fisher linear functions which characterize each one of the three possible genotypes:

AA	Function 1
AB	Function 2
BB	Function 3

The function which presents the highest absolute value determines the genotype of the patient.

The Fisher linear functions are obtained by analyzing 3 subjects for each of the three possible genotypes of the genetic variation (AA, AB, BB). With the results, ratios 1 and 2 are calculated for the SNPs analyzed and for the 3 subjects. These ratios are classification variables for the three groups to create the linear functions, with which the discriminatory capacity of the two pairs of designed probes is evaluated. If the discriminatory capacity is not 100%, the probes are redesigned. New subjects characterized for each of the three genotypes make up new ratios 1 and 2 to perfect the linear functions and in short, to improve the discriminatory capacity of the algorithm based on these three functions.

When using a fluorescent laser, to obtain reliable results it is preferable that ratios 1 and 2 are within the range of the ratios used to build the groups.

Again when a fluorescent scanner is used in the experiment, for a complete hybridization to be considered reliable preferably the ratio of probe fluorescence intensity to background noise of all the beads DNA array probes is above 15. Likewise, the average of all the ratios is preferably above 0.6 and the negative control is preferably less than or equal to 3 times the background noise.

In summary, four probes are presented in the hybridization analysis for detection of each mutation. Two of the probes detect one genetic variation (A) and the other two the other genetic variation (B). The examined base is located in the central position of the probes.

A subject homozygous for the genetic variation A will not show genetic variation B. Consequently, the probes which detect genetic variation B will show a hybridization signal significantly less than that shown by variation A and vice versa. In this case the ratios 1 and 2 will show 1 and the subjects will be assigned as homozygous AA by the software analysis.

On the other hand, a heterozygous subject for the determined genetic variation shows both the genetic variations. Therefore, the probes which detect them show an equivalent hybridization signal. The ratios 1 and 2 will show 0.5 and the subject will be assigned as heterozygous AB the software analysis as described.

In one aspect of the invention, DNA polymorphisms are selected based on their association with the etiology of MS, such as those shown in Table 1 below:

Table 1

Gene	RefSNP accession I.D.
ACCN1	rs28936
ACE	rs4343
ADAMTS14	rs4747075
ADAMTS14	rs7081273
ADAMTS14	rs4746060
ALK	rs7577363
ANKRD15	rs10975200
Apo I/Fas	rs1800682
Apo I/Fas	rs3781202
Apo I/Fas	rs2234978
BDNF	rs6265
BTNL2	rs2076530
C10orf27	rs2254174
C10orf27	rs12221473
C10orf27	rs12221474
C10orf27	rs2791196
CACNG4	rs4790896
CBLB	rs12487066
CCL11	rs17735961
CCL14	rs854682
CCL17	rs223828
CCL2	rs1024610
CCL22	rs4359426
CCL23	rs1003645
CCL23	rs854655
CCL5	rs2107538
CCL5	rs2280788
CCR5	rs333
CD14	rs2569190
CD226	rs763361
CD24	rs8734
CD58	rs12044852
CNTF	rs1800169
CRYAB	rs14133
CRYAB	rs762550
CRYAB	rs2234702
CTLA4	rs231775
CTLA4	rs5742909
CTSS	rs1136774
CTSS	rs3754212
CXLCL10	rs3921

Gene	RefSNP accession I.D.
CXLCL10	rs8878
DBC1	rs10984447
DRB1	rs3135388
EBF	rs1368297
EVI5	rs10735781
EVI5	rs6680578
FAM69A	rs11164838
FAM69A	rs7536563
GABBRA1	rs1805057
GLO1	rs2736654
GR	rs6189
GR	rs6190
HELZ	rs2363846
HFE	rs1800562
HLA	rs2395166
HLA	rs2213584
HLA	rs2227139
HLA	rs3135388
HLA	rs9268458
HLA	rs6457594
HLA-DRA	rs2395182
HLA-DRA	rs2239802
ICOS	rs4404254
ICOS	rs10932036
ICOS	rs4675379
IFI30	rs11554159
IFNAR	rs1012334
IFNAR1	rs2257167
IFNG	rs1861494
IFNG	rs2069727
IFNG	rs2430561
IFNG	rs3181034
IFNG	rs7954499
IFNGR2	rs9808753
IKBL	rs3130062
IL10	rs1800871
IL10	rs1800872
IL10	rs1800896
IL1A	rs1800587
IL1B	rs1799916
IL1B	rs1143627
IL1B	rs1143634
IL1RN	2073 Intron2 C/T (rs423904)
IL1RN	rs419598
IL2	rs2069763

Gene	RefSNP accession I.D.
IL2	rs2069762
IL23R	rs7517847
IL23R	rs11209026
IL2RA	rs12722489
IL2RA	rs2104282
IL4R	rs1801275
IL5RA	rs2290608
IL7R	rs11567685
IL7R	rs7718919
IL7R	rs11567686
IL7R	rs6897932
IL7R	rs3194051
IL7R	rs987106
IL7R	rs987107
IL7R	rs11567685
IL7R	rs7718919
IL7R	rs11567686
IL8	rs4073
IRF1	rs2070721
IRF5	rs3807306
IRF5	rs4728142
IRF5	5 bp insertion-deletion polymorphism located in the promoter and first intron of the IRF5 gene
IRF-5	rs10954213
IRF-5	rs2004640
IRF-5	rs2280714
IRF-5	rs3757385
ITGA4	rs1449263
KCNH7	rs2068330
KIAA0350	rs6498169
KLC1	rs8702
KLRB1	rs4763655
LAG3	rs1922452
LAG3	rs870849
LAG3	rs951818
LAG3	rs19922452
LMP7	rs2071543
MBP	rs470929
MC1R	rs1805009
MC1R	rs1805006
MEFV	rs28940577
MGC33887	rs987931
MHC2TA	rs4774
MHC2TA	rs3087456

Gene	RefSNP accession I.D.
MOG	rs2857766
MOG	rs3130250
MOG	rs3130253
MxA	rs2071430
NDUFA7	rs2288414
NDUFA7	rs561
NDUFS5	rs2889683
NDUFS5	rs6981
NDUFS7	rs2074897
NOS2A	rs1137933
NOS2A	rs2779248
NOTCH4	rs367398
NR4A2	rs1405735
OAS1	rs10774071
OAS1	rs3741981 (rs1131454 in version. 37.1)
PD-1	rs11568821
PDE4B	rs1321172
PITPNC1	rs1318
PITPNC1	rs2365403
PNMT	rs876493
PON	rs854560
PPARG	rs1801282
PRKCA	rs7220007
PRKCA	rs887797
PRKCA	rs2078153
PRKCA	rs3890137
PTPN22	rs2476601
PTPRC	rs17612648
PTPRC	rs4915154
PVRL2	rs394221
RPL5	rs6604026
SELE	rs1805193
SELE	rs5361
SPARCL1	rs1049544
Spp1	rs1126616
Spp1	rs1126772
Spp1	rs2853744
Spp1	rs9138
Spp1	rs4754
STAT1	rs1547550
STAT1	rs2066802
TAC1	rs2072100
TAC1	rs7793277
TGFB1	rs1800469
TGFB1	rs1800470

Gene	RefSNP accession I.D.
TGFB1	rs1800471
TGFB1	rs1982073
TNF-alpha	rs1800629
TRAIL	rs1131568
TRIF (TICAM1)	rs1046673
TRIF (TICAM1)	rs2292151
UCP2	rs659366
VDR	rs10735810
VDR	rs1544410
VDR	rs731236

Each individual in the study population is tested to determine an outcome for each of the discriminating variables for the particular phenotype. This provides a number of outcomes for each individual. Testing, e.g. genotyping, may be carried out by any of the methods described herein, e.g. by microarray analysis as described herein. Testing is typically ex vivo, carried out on a suitable sample obtained from an individual.

Multiple genotype-phenotype associations may then be analysed using stepwise multivariate logistic regression analysis, using as the dependent variable the clinically determined MS phenotype and as independent variables the outcomes of the informative variables. The goodness of fit of the models obtained may be evaluated using Hosmer-Lemeshow statistics and their accuracy assessed by calculating the area under the curve (AUC) of the Receiver Operating Characteristic curve (ROC) with 95% confidence intervals (see, e.g. (Janssens ACJW et al., 2006)).

Mean probability function values for each of the alternative phenotypes in the population can be compared using a t test. In general the probability functions are able to distinguish between the different phenotypes in the study population in a statistically significant way, for example, at $p \leq 0.05$ in a t-test. Thus the probability functions produce a statistically significant separation between individuals of different phenotype in the population.

In some embodiments, the presence of two or more genetic markers in a sample from an MS patient is compared to the presence of two or more genetic markers in a control sample. In some embodiments a control sample is a sample from an individual who does not have MS. In other embodiments a control sample is a sample from an individual who

has MS. In certain embodiments, a control sample is a sample from an individual who has MS of a specified classification or degree of severity. It will be understood that the interpretation of a comparison between a test sample and a control sample will depend on the nature of both samples. One possible measurement of the level of expression of genetic markers in a sample is the absolute number of genetic markers identified in a sample. Another measurement of the level of expression of genetic markers in a sample is a measurement of the specific combination of genetic markers in a sample.

In some embodiments, a control value may be a predetermined value, which can take a variety of forms. It can be a single cut-off value, such as a median or mean. It can be established based upon comparative groups, such as in groups not having MS, or groups have specified classifications or levels of severity of MS. For example, in some embodiments, a control sample that is taken from an individual who does not have MS, may be considered to exhibit control or normal patterns of expression of genetic markers for MS. In some embodiments where severity of MS is being assessed, a control sample that is taken from an individual that has a specified classification or level of severity of MS, such as a mild form of MS, may be considered to exhibit a normal or control pattern of expression of markers for MS. In some embodiments a control sample will be from an individual who is of the same ethnic background, gender, age, MS classification and/or MS disease duration as the individual who is being screened and/or diagnosed.

Based at least in part on results of correlations and methods discussed herein, predetermined values can be arranged. For example, test samples and subjects from which the samples were extracted can be divided into groups such as low-severity, medium-severity, and high-severity groups based on the presence of two or more genetic markers that are correlated to MS severity. In some embodiments the classification of a sample and subject into a group can be used to aid or assist in screening, diagnosis, prognosis or development of a treatment strategy for a given subject.

Described herein are correlations between the presence of specific genetic markers and the severity of symptoms of MS in a patient. Such correlations and methods for detecting such correlations have widespread applications for MS patients. In some embodiments methods described herein are used to screen patients who have or are at risk of having MS. In some embodiments, evaluation of the presence of two or more SNPs in a

patient will be used to assist in the diagnosis or to indicate or evaluate the severity of MS in the patient. In some embodiments, genetic information obtained from methods described herein will be combined with other clinical data to assess the severity of MS in a given patient.

In some embodiments, the identification of two or more SNPs in a DNA sample from an MS patient will be used to initiate or change a treatment regimen for the patient. For example, in some embodiments, detection of two or more SNPs that are associated with increased severity of MS may cause a physician to change the treatment strategy of an MS patient in order to target a more severe form of the disease, or advise a patient that they may benefit from a change in treatment strategy. In some embodiments, detection of two or more SNPs that are associated with increased severity of MS may cause a physician to monitor an MS patient more closely or rigorously. In some embodiments, detection of two or more SNPs that are associated with increased severity of MS may cause a physician to recommend or advise that a patient undergo genetic counselling.

In some aspects of the invention measurement of clinical variables comprises part of the severity prediction model along with the genetic variables in Table 1, above. Some non-limiting examples are age at onset, gender of patient studied, and type of onset of the disease (e.g. progressive or relapsing) (see Table 2). Age at onset refers to the age in years at which the patient was diagnosed with MS. In the present models this measure has been treated as a continuous variable, which is included in the logistic regression function of the models. Thus an outcome for this variable is age of patient when diagnosed for MS.

Gender refers to the gender of the patient diagnosed with MS. In the present models this measure has been treated as a categorical variable, with levels "male" and "female", which is included in the logistic regression function of the models. Thus an outcome for this variable is gender of patient diagnosed with MS. If the gender is male, this is coded as (1), and if the gender is female, this is coded as (0).

Type of onset refers to the type of onset of disease, progressive or relapsing, for the studied patient diagnosed with MS. In the present models this measure has been treated as a categorical variable, which is included in the logistic regression function of the models. Thus an outcome for this variable is age of patient when diagnosed for MS. If the type of

onset is progressive, this is coded as (1), and if the type of onset is relapsing, this is coded as (0).

Table 2: Clinical Variables

Variable	Variable Type
Age at onset (Age_at_onset)	Continuous variable
Gender	Categorical variable
Onset type (Onsettype_cod)	Categorical variable

In embodiments comprising methods of evaluation of MS severity in a patient, the method typically comprises determining or obtaining for the subject, an outcome for each of the variables listed in Table 2. In some embodiments, use of the results of the measurements of these variables, along with the variables in Table 1, allows prognosis of MS severity phenotype in a Dutch population with an LR+ of 8.4. Details for the calculation of a probability function using these variables are given in Table 3.

Preferably the number and combination of variables such as SNPs used to construct a model for predicting a phenotype according to the invention, is such that the model allows prediction to be made with an LR+ value of at least 1.5, such as at least 2, 3, 4, 5, 6, 7, 8, 9, or 10. Calculation of LR+ values is described herein.

Once an outcome is determined for each of the variables for prediction of a given phenotype, these outcomes are used in or inserted in a suitable probability function (for prediction of that phenotype), as described herein and a probability function value is calculated. Outcomes may be codified for use in the probability function and calculation of the probability function value. The probability function value is then compared with probability function values obtained for a population of individuals of known (clinically determined) phenotype. The risk of the subject having or developing the particular phenotype is thereby determined.

The sensitivity, specificity, and positive likelihood ratio (LR+ = sensitivity/(1-specificity)) may be computed by means of ROC curves. Preferably the model has an LR+ value of at least 1.5, for example, at least 2, 3, 4, 5, 6, 7, 8, 9 or 10.

Also within the scope of the invention are kits and instructions for their use. In some embodiments kits associated with the invention are kits for identifying two or more SNPs

within a patient sample. In some embodiments a kit may contain primers for amplifying a specific genetic locus. In some embodiments, a kit may contain a probe for hybridizing to a specific SNP. A kit of the invention can include a description of use of the contents of the kit for participation in any biological or chemical mechanism disclosed herein. A kit can include instructions for use of the kit components alone or in combination with other methods or compositions for assisting in screening or diagnosing a sample and/or determining a treatment strategy for MS.

The kits described herein may also contain one or more containers, which may contain a composition and other ingredients as previously described. The kits also may contain instructions for mixing, diluting, and/or administering or applying the compositions of the invention in some cases. The kits also can include other containers with one or more solvents, surfactants, preservative and/or diluents (e.g., normal saline (0.9% NaCl), or 5% dextrose) as well as containers for mixing, diluting or administering the components in a sample or to a subject in need of such treatment.

The compositions of the kit may be provided as any suitable form, for example, as liquid solutions or as dried powders. When the composition (e.g., a primer) provided is a dry powder, the composition may be reconstituted by the addition of a suitable solvent, which may also be provided. In embodiments where liquid forms of the composition are used, the liquid form may be concentrated or ready to use. The solvent will depend on the composition and the mode of use or administration. Suitable solvents for drug compositions are well known, for example as previously described, and are available in the literature. The solvent will depend on the composition and the mode of use or administration.

As used herein, the term "subject" refers to a human or non-human mammal or animal. Non-human mammals include livestock animals, companion animals, laboratory animals, and non-human primates. Non-human subjects also specifically include, without limitation, chickens, horses, cows, pigs, goats, dogs, cats, guinea pigs, hamsters, mink, and rabbits. In some embodiments of the invention, a subject is a patient. As used herein, a "patient" refers to a subject who is under the care of a physician or other health care worker, including someone who has consulted with, received advice from or received a prescription or other recommendation from a physician or other health care worker. A patient is typically a subject having or at risk of having MS.

The term "treatment" or "treating" is intended to relate to prophylaxis, amelioration, prevention and/or cure of a condition (e.g., MS). Treatment after a condition (e.g., MS) has started aims to reduce, ameliorate or altogether eliminate the condition, and/or its associated symptoms, or prevent it from becoming worse. Treatment of subjects before a condition (e.g., MS) aims to reduce the risk of developing the condition and/or lessen its severity if the condition does develop. As used herein, the term "prevent" refers to the prophylactic treatment of a subject who is at risk of developing a condition (e.g., MS) resulting in a decrease in the probability that the subject will develop the disorder, and to the inhibition of further development of an already established disorder.

Treatment for MS varies with the stage of the disease and the clinical presentation of the patient. In general it is advantageous to begin treatment early in the course of the disease. Goals for treatment may include slowing the progression of the disease, reducing the number of the attacks, and improving recovery from attacks. Corticosteroids such as Methylprednisolone (Solu-Medrol®, Medrol, Depo-Medrol), and Prednisone (Deltasone ®, Liquid Pred, Orasone, Prednicen-M) are used to treat exacerbations of MS. In some embodiments Methylpredisone is given intravenously for 2-7 days, followed by a course of Prednisone. Corticosteroids may be used only for very severe attacks, as the effects vary and there are numerous reported side effects.

In some embodiments an MS patient is treated with therapies that can modify the course of the disease. Certain immune modulatory therapies are thought to slow the progression of MS by tempering the immune system's attack on the central nervous system. Some non-limiting examples include Interferon beta-1a, Interferon beta-1b, and Glatiramer acetate. Some examples of Interferon beta-1a include Avonex® and Rebif®. Avonex® is typically administered by intramuscular injection once weekly, whereas Rebif® is typically administered subcutaneously 3 times per week, at a dose of 22 or 44 mcg. Interferon beta-1b, e.g. Betaseron, is in some embodiments given by subcutaneous injection ever other day. Patients treated with interferon may experience fewer relapses or faster recovery from attacks, and an overall slowing of the progression of the disease. Glatiramer acetate, e.g. Copaxone®, is a synthetic amino acid that modifies actions of the immune system that may affect the progression of MS. It has been shown to reduce the frequency of exacerbations

and the level of disability. In some embodiments this medication is given subcutaneously daily.

Other immune modulatory therapies include Natalizumab (Tysabri®), a monoclonal antibody against VLA-4, Mitoxantrone (Novantrone®), a chemotherapy drug. Natalizumab is administered via monthly intravenous injections and has been shown to reduce the frequency of clinical relapses and delay the progression of physical disability. Mitoxantrone is used for reducing neurologic disability and/or the frequency of clinical relapses. In some embodiments vitamin D is used as a treatment.

Other treatments for relief from complications of the disease are aimed at specific symptoms, such as muscle spasticity, weakness, eye problems, fatigue, emotional outbursts, pain, bladder dysfunction, constipation, sexual dysfunction, and tremors.

EXAMPLES

In multiple sclerosis (MS), the total volume of spinal and brain lesions and their spatial distribution are highly variable. Elucidating this variability may contribute to understanding clinical heterogeneity in MS.

Materials and Methods:

Study participants:

Patients were selected retrospectively from natural history studies conducted at the MS Center at the VU University Medical Center in Amsterdam. Patients were selected for the availability of DNA material, as well as spinal cord and brain MRI, which fulfilled certain standardization requirements and were performed less than two years apart. The study was carried out with the approval of the Medical Ethical Committee of the VUmc and informed consent was obtained from all participants. Patients, all diagnosed with MS ascertained by Poser or McDonald criteria (Poser et al., Ann. Neurol 1983;3:227-231; Polman CH et al., Ann. Neurol. 2005;6:840-846). For the patients included in the analysis, clinical data were collected including age, gender, type of disease onset, age at onset, disease course and duration of disease. Disability status was determined for all subjects by using Kurtzke's Expanded Disability Status Scale (EDSS) and when available Multiple Sclerosis Functional Composite scale (MSFC).

Selection of SNPs:

Polymorphisms were selected based on published involvement in MS pathogenesis, prognosis and response to treatment. The polymorphisms were confirmed and associated to an identifier by using dbsnp database (www.ncbi.nlm.nih.gov/SNP). Nucleotide sequences for the design of allele-specific probes and PCR primers where retrieved in the SNPper database (http://snpper.chip.org/bio). Sequence specific probes and primers were designed by using the software Primer3 freely available at http://frodo.wi.mit.edu/. Some non-limiting examples of probes and primers useful in the instant invention can be found in Tables 7-9.

If a polymorphism was not present in the database, position and sequences were established by performing a blast search (http://www.ncbi.nlm.nih.gov.catalog.llu.edu/BLAST/) using the data available in the literature.

Genotyping

Genomic DNA was isolated from anti-coagulated blood with DNAzol reagent (Molecular Research Center, Inc., Cincinnati, OH).

Genotyping was carried out using a newly developed low-density DNA microarray based on allele-specific probes. The design, fabrication, validation and analysis of the arrays were performed following the procedure described by Tejedor *et al.* (2005), *Clin. Chem.*, Vol. 51(7), pp. 1137-1144, with minor modifications.

Brain MRI

Scans were acquired either on 1.0 Tesla or 1.5 Tesla (Siemens) scanners with standard head coils, using standard 2D conventional or fast spin-echo PD- and T2-weighted images (TR: 2200-3000 ms, TE: 20-30 & 80-100 ms) with a slice thicknesses of 3-5 mm, a maximum gap between slices of 0.5 mm, and an in-plane resolution of 1x1 mm^2 . Lesions were identified by an expert reader and then outlined on the corresponding PD image using home-developed semi-automated seed-growing software based on a local thresholding

technique. Lesion areas were multiplied with the interslice distance to obtain total T2 brain lesion volume for each patient.

Spinal cord MRI:

Spinal cord scanning included a cardiac-triggered sagittal PD and T2-weighted dual-echo spin echo sequence with a slice-thickness of 3mm covering the whole spinal cord (TR: 2500 – 3000 ms, TE: 20-30 & 80-100 ms) with an in plane resolution of 1x1 mm. From this sequence the number of focal abnormalities and the presence of diffuse abnormalities were scored by one experienced reader (CL). Diffuse abnormalities were defined as areas with poorly delineated areas of increased signal intensity compared to signal intensity of spinal CSF on intermediate-weighted images.

Statistical methods for MRI data:

First the association between the brain parameter (T2 lesion load) and spinal cord parameters (number of focal lesions, presence of diffuse abnormalities) were tested per SNP and per clinical variable. The non-parametric Kruskal-Wallis test and ChiSquare test were used appropriately, applying the False Discovery Rate (FDR) according to Benjamini and Hochberg (Benjamini, Y, 1995, J.R. Stat. Soc. B Met 289) to correct for multiple testing. The corrected number represents the expected proportion false discoveries for a given p-value cut-off. The cut-off point after FDR correction of $p<0.05$ was used. Pearson's correlation coefficient was used to test the correlations between two scaled variables. All analyses were performed using SPSS (version 15; SPSS Inc., Chicago, IL, USA).

Statistical methods for regression and association analysis:

First the association between MS severity score, the brain parameter (T2 lesion load) and spinal cord parameters (number of focal lesions, presence of diffuse abnormalities) were tested per SNP and per clinical variable and statistically significant associations between particular genotypes and particular phenotypes are identified. Methods for determining statistical significance are known in the art. Models were created by means of multivariate logistic and/or linear regression, for categorical or continuous dependent variables respectively, with clinically determined disease phenotypes as dependent variables and the

SNPs and clinical variables as independent variables or regressors. To evaluate the impact of the regressors included in the prognosis of the analysed phenotypes, the sensitivity, specificity and positive likelihood ratio (LR+ = sensitivity/(1-specificity)) were computed by means of Receiver Operating Characteristic curves. In the case of multiple linear regression, the impact of the regressors the corrected R square was computed. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15 and HelixTree (Golden Helix, Inc., Bozeman, MT).

Example 1: Identification of polymorphisms associated with increased MSSS score

The invention presents a model for predicting the probability of having a stronger disability, as measured by the MSSS scale, thus allowing differential treatment management for a given patient. This model was obtained from the analysis of 605 MS patients. The invention evaluates differences between patients that have an MSSS score of less than 2.5 versus patients that have an MSSS score of 2.5 or greater.

Table 3 (shown below) shows the six SNPs (rs876493, rs1137933, rs1318, rs2069763, rs2107538 and 2073 Intron2 C/T (rs423904)) with the associated genotypes and the three clinical variables (age at onset, gender and onset type) and the associated levels, together with their significance (Sig.), the coefficients in the model (B) and their odds ratios (OR) with lower and upper bound confidence intervals (I.C 95.0% for OR) used to compute the model for the prediction of the MSSS<2.5 versus ≥2.5 phenotype. This model provides the probability to develop a severe form of MS.

Table 3: Regression Analysis

Variable name	Genotype/Variable level	Sig.	B	OR	I.C. 95% for OR	
					Lower bound	Upper bound
IL1RN 2073 Intron2 C/T (rs423904)	CC vs CT/TT	0.064	-0.469	0.625	0.381	1.028
PNMT rs876493	AA/AG vs GG	0.025	-0.65	0.522	0.295	0.923
Age_at_onset		0.004	0.048	1.049	1.016	1.083
gender	0=female vs 1=male	0.017	0.684	1.982	1.127	3.485
Onsettype_cod	0=relapsing vs 1=progressive	0.021	1.466	4.331	1.244	15.082
NOS2A rs1137933	GG vs AG/AA	0.005	-0.715	0.489	0.298	0.803
PITPNC1 rs1318	AA vs AG/GG	0.002	-0.775	0.461	0.28	0.759
IL2 rs2069763	GG vs GT/TT	0.001	-0.922	0.398	0.23	0.688
CCL5 rs2107538	CC vs CT/TT	0.023	0.649	1.914	1.092	3.355

Figure 1 shows a ROC (receiver operating characteristic) curve obtained for the model MSSS<2.5 versus ≥2.5 that allows the estimation of its discriminatory power. The ROC curve was calculated in order to maximize the specificity, thus reducing at the same time the "false" positive rate. A specificity of 95.3% with a sensibility of 39.7% is the cut-off for this model regarding the phenotype MSSS<2.5 versus ≥2.5. This model shows a positive likelihood ratio (LR+) value of 8.4.

Additional MS patients have been recruited increasing the MS cohort to 700 MS patients. In a first stage of analysis, feature selection was employed to identify the most important and predictive features in the model to be analyzed. This approach of variable filtering is based on the marginal association between each variable (SNP or clinical variable) and phenotype, as variables are typically filtered on the basis of a p-value cut-off from a univariate analysis. For the selection of variables, HelixTree® software (Golden Helix, Inc., Bozeman, MT, USA) was used to calculate allelic association between different groups. In Table 3A, SNPs associated with MSSS score at a significance level of p<0.1 set as the decision threshold are shown.

Table 3A: Table showing the 37 SNPs associated with MSSS score at the selected significance level

Rs-number SNP	Gene	Sig. p-value
rs3756450	LOC728594	0,00436
rs12047808	C1orf125	0,00883
rs10259085	C1GALT1	0,00949
rs1042173	SLC6A4	0,01142
rs1318	PITPNC1	0,01426
rs6077690	SNAP25	0,02478
rs1611115	DBH	0,02577
rs2107538	CCL5	0,03258
rs4473631	MORF4	0,03348
rs2032893	SLC1A3	0,03470
rs2066713	SLC6A4	0,03744
rs260461	ZNF544	0,03924
rs3787283	SNAP25	0,03976
rs1137933	NOS2A	0,04094
rs6917747	IGF2R	0,04710
rs2049306	CSMD1	0,04909
rs12861247	STS	0,05177
rs4404254	ICOS	0,05585
rs4680534	IL12A	0,05729
rs17641078	DMRT2	0,05833
rs2187668	HLA-DQA1	0,06045
rs7528684	FCRL3	0,06099
rs876493	PNMT	0,06135
rs7577925	FLJ34870	0,06232
rs1805009	MC1R	0,06375
rs423904	IL1RN	0,06449
rs3741981 (rs1131454 in version. 37.1)	OAS1	0,06993
rs2069763	IL2	0,07750
rs12202350	IGF2R	0,07981
rs28386840	SLC6A2	0,08145
rs2028455	LOC647094	0,08244
rs10492503	GPC5	0,08486
rs8049651	GRIN2A	0,08826
rs13353224	DSEL	0,08906
rs1555322	MMP24	0,09161
rs10243024	MET	0,09398

Rs-number SNP	Gene	Sig. p-value
rs6570426	LOC729293	0,09635

A Multivariate prognostic model was then constructed for dichotomous MSSS with the cut-off point of 2.5 using logistic regression model, using SPSS version 15.0 (SPSS Inc. Headquarters, Chicago, IL, USA) and R packages Design (Harrell, 2001) and Stats (R Development Core Team, 2008). The model was developed including information for the clinical variables available.

85% of the cohort was selected at random as exploratory cohort (n=595) and the 15% of the cohort as replication cohort (n=105). The model obtained with the exploratory cohort (Table 3B) included the same variables as the one obtained from the analysis of 605 MS patients (Table 3). The model showed in Table 3B was validated in the replication cohort (AUC exploratory cohort=0.743 (0.691-0.796) (Figure 5) versus AUC replication cohort=0.787 (0.667-0906) (Figure 6), differences between both ROC curves not statistically significant).

Table 3B: Regression Analysis

					I.C. 95% for OR	
Variable name	Genotype/Variable level	Sig.	B	OR	Lower bound	Upper bound
IL1RN 2073 Intron2 C/T (rs423904)	CC vs CT/TT	0.056	-0.482	0.618	0.377	1.012
PNMT rs876493	AA/AG vs GG	0.071	-0.531	0.588	0.331	1.046
Age_at_onset		0.069	0.028	1.029	0.998	1.061
gender	0=female vs 1=male	0.01	0.743	2.102	1.194	3.703
Onsettype_cod	0=relapsing vs 1=progressive	0.006	1.71	5.529	1.616	18.917
NOS2A rs1137933	GG vs AG/AA	0.018	-0.593	0.553	0.339	0.901
PITPNC1 rs1318	AA vs AG/GG	0.026	-0.561	0.571	0.349	0.934
IL2 rs2069763	GG vs GT/TT	0.009	-0.709	0.492	0.288	0.839
CCL5 rs2107538	CC vs CT/TT	0.031	0.606	1.832	1.058	3.173

The model obtained from the analysis of the 700 MS patients is showed in Table 3C. The model includes the same variables that the obtained from the analysis of 605 MS patients (Table 3) and from the analysis of the exploratory cohort or 595 MS patients (Table 3B).

Table 3C: Regression Analysis

Variable name	Genotype/Variable level	Sig.	B	OR	I.C. 95% for OR Lower bound	Upper bound
IL1RN 2073 Intron2 C/T (rs423904)	CC vs CT/TT	.084	-.404	.668	.422	1.056
PNMT rs876493	AA/AG vs GG	.053	-.533	.587	.342	1.006
Age_at_onset		.015	.036	1.036	1.007	1.066
gender	0=female vs 1=male	.017	.634	1.884	1.119	3.173
Onsettype_cod	0=relapsing vs 1=progressive	.005	1.758	5.801	1.714	19.638
NOS2A rs1137933	GG vs AG/AA	.005	-.649	.522	.331	.824
PITPNC1 rs1318	AA vs AG/GG	.025	-.527	.590	.373	.935
IL2 rs2069763	GG vs GT/TT	.001	-.818	.441	.267	.730
CCL5 rs2107538	CC vs CT/TT	.015	.643	1.902	1.132	3.196

The ROC curve area obtained for the model MSSS≥2.5 vs MSSS<2.5 analysing the 700 MS patients is 0.749 (95%CI 0.700-0.797) (Figure 7). A specificity of 95% with a sensitivity of 32% is the cut-off for this model. The model shows a positive likelihood ratio (LR+) value of 6.2.

Example 2: Identification of SNPs associated with T2 brain lesions

In order to determine whether certain SNPs are associated with increasing size and distribution of T2 brain lesions, analysis was performed on a group of 208 MS patients with MRI data collected. The MRI data show spatial distribution of T2 brain lesions. Figure 2 shows lesion frequency across the patient sample.

Figure 3 shows maps of clusterwise (t=2) associations of lesion presence with genotype, on a background of the common brain image. The cluster colour bar indicates clusterwise p-value, with the range indicated by the colour bar; only clusters with $p<0.05$ are shown. These data have been correlated to genotype data. The results show significant associations for four SNPs to brain lesions. A: rs2213584 (HLA-DRA gene); B: rs2227139 (HLA-DRA gene); C: rs2076530 (BTNL2 gene); D: rs876493 (PNMT gene).

Example 2A: Identification of SNPs associated with T2 brain lesions

Further investigation was carried out essentially as described in Example 2. Additionally, lesions were manually outlined on Magnetic Resonance Imaging scans and binary lesion masks were produced and registered to a common space. Using Randomise software, the lesion masks were related to genotype using a voxelwise nonparametric General Linear Model approach, followed by clusterwise analysis. The results show significant associations for eight SNPs to brain lesions: rs9808753 (IFNGR2 gene), rs2074897 (NDUFS7 gene), rs762550 (CRYAB gene), rs2076530 (BTNL2 gene), rs2234978 (FAS gene), rs3781202 (FAS gene), rs2107538 (CCL5 gene), rs659366 (UCP2 gene).

Example 3: Identification of SNPs associated with MS severity phenotypes

Patient characteristics:

One hundred and fifty patients were included in the analysis. The patient group reflects a representative MS population, with approximately 35% men and 20% primary progressive MS patients (see Table 4). The majority of patients (132/150) demonstrated abnormalities on the spinal cord MRI scan, while all patients had abnormalities on the brain MRI scan.

Table 4: Patient characteristics

	All	RR	SP	PP
Total n	150	88	32	30
Gender (n; % M)	55 (36.7%)	26 (29.5%)	17 (53.1%)	12 (40%)
Age at MRI (mean)	41.4	36.1	46.5	51.2
Disease duration mean (range)	7.1 (0.0-33.0)	4.36 (0.0-32.0)	12.8 (2.0-33.0)	9.2 (0.0-28.0)

	All	RR	SP	PP
EDSS (median)	3.5	2.0	5.5	4.0
T2 lesionload (ml) (mean)	7.7	4.9	16.2	7.0
Number of focal lesions in the spinal cord (mean)	3.4	3.3	4.5	2.8
Percentage of patients with diffuse abnormalities (%)	13.3	10.2	18.8	16.7

Genotyping:

In total 80 SNPs in 44 genes were selected on the MSchip. Twelve SNPs were excluded from further analysis: five were monomorphic and seven SNPs had a minor allele frequency below five percent (see Table). Hardy Weinberg equilibrium was calculated for all SNPs. Values are noted in table 5.

Table 5: Results analysis of the correlation SNPs and MRI parameters. * and ** LD values still need to be calculated.

Clinical / MRI parameter correlated with SNPs:	Rs-number SNP	Gene	Uncorrected p-value Kruskal Wallis test:	FDR-corrected p-value
Number of focal lesion in the spinal cord	rs3135388	MHC II	0.00082	0.03
	rs2395182 *	MHC II	0.00107	0.03
	rs2239802*	MHC II	0.00122	0.03
	rs2227139**	MHC II	0.00169	0.03
	rs2213584**	MHC II	0.00330	0.05
	rs3087456	MHC II TransActivator	0.00900	0.10
T2 lesion load in the brain	rs2107538	CCL5	0.001	0.07

Correlation between clinical parameters and MRI features:

The EDSS showed a mild correlation with the number of focal lesions in the spinal cord ($p= 0.043$, $r= 0.165$, Pearson correlation), with the number of segments involved ($p=0.006$, $r=0.224$, Pearson correlation) and a moderate correlation with T2 lesion load in the brain ($p<0.001$, $r=0.395$). A weak correlation was present between the number of focal spinal cord lesions and T2 lesion load in the brain ($p=0.063$, $r=0.152$).

Disease duration was found to be related to number of segments of the spinal cord involved ($p=0.017$, $r=0.195$).

The T2 lesion load in the brain was closely related to the PASAT score ($p= 0.000$, $r= -0.581$) and 9 Hole Peg Test of the dominant hand ($p=0.001$, $r=0.306$).

Correlation between lesion load in the brain and genotypes:

In the univariate analysis on T2 lesion load in the brain and the MS-chip, the only 'trend' correlation was rs2107538 (CCL5) (see Table 5).

Correlation between spinal cord abnormalities and genotypes:

Several HLA SNPs were found to be related to the number of focal spinal cord abnormalities (see Table 5). The most significant is SNP rs3135388. Carriership of the A-allele (surrogate marker for HLA-DRB1*1501) was associated with a significantly higher number of lesions in the spinal cord (Figure 4).

When corrected for multiple testing, five SNPs within the MHC region (rs3135388, rs2395182, rs2239802, rs2227139 and rs2213584), remained significant and one SNP within the MHC-2TA gene (Major Histocompatibility Complex Class II Transactivator) showed a trend towards a correlation. The five HLA SNPs are in high linkage disequilibrium.

A linear model has been developed using multiple linear regression to predict the number of focal lesions in the spinal cord. This method uses three of these SNPs rs3135388, rs3087456, and rs2227139. A model including the combination of these three SNPs improves the use of one single SNP (rs3135388) for prediction of number of focal lesions in the spinal cord. Corrected Rsquared for model using only one SNP = 0.064. Corrected Rsquared for model using combination of three SNPs = 0.112. The combination

of these three SNPs or any SNP in linkage disequilibrium with any of these three SNPs improves prediction of number of focal lesions in the spinal cord over the use of one single SNP.

No interactions between the SNPs and the clinical variables were present. No association was observed between the presence of diffuse abnormalities and the evaluated SNPs.

Example 4: Identification of additional SNPs associated with MRI parameters: Number of focal lesion in the spinal cord, T2 lesion load in the brain and Presence of diffuse abnormalities

In order to determine whether certain additional SNPs are associated with MRI parameters, a similar analysis to Example 3 was performed using different SNPs on one hundred and fifty patients. Results of the correlation of additional SNPs and MRI parameters are shown in table 5A.

In our study cohort of 150 MS patients with MRI data, MRI data are significantly correlated with MS severity given by MSSS ($p= 0.023$). It can thus be assumed that identification of SNPs associated with MRI parameters allows inferring MS severity.

Table 5A: Results of analysis of the correlation of additional SNPs and MRI parameters.

Clinical / MRI parameter correlated with SNPs:	Rs-number SNP	Gene	Uncorrected p-value Kruskal Wallis test:
Number of focal lesion in the spinal cord	rs10492972	KIF1B	0.0063
	rs12202350	IGF2R	0.005
	rs8049651	GRIN2A	0.0023
	rs8702	KLC1	5.00E-04
	rs987107	IL7R	0.0091
T2 lesion load in the brain	rs12861247	STS	0.005
	rs2074897	NDUFS7	0.006
	rs7995215	GPC6	0.006
Presence of diffuse abnormalities	rs1350666	EREG	0.008
	rs3808585	ADRA1A	0.003
	rs4128767	IL16	0.006
	rs6457594	MHC II	0.005
	rs7208257	ARRB2	0.006
	rs7956189	NTF3	0.008

Table 6: SNPs included in the analyses; HWE=Hardy-Weinberg Equilibrium in our sample; MAF=minor allele frequency in our sample

Gene	rs-nr	Chromosome	Poly-morphism	HWE *	MAF
ADAMTS14	rs4747075	10q22	A/G	7.74*	0.30
ADAMTS14	rs7081273	10q22	C/G	1.2	0.34
ADAMTS14	rs4746060	10q22	C/T	1.05	0.08
Apo I/Fas	rs1800682	10q23	C/T	0.02	0.47
Apo I/Fas	rs3781202	10q23	C/T	7.41 *	0.40
Apo I/Fas	rs2234978	10q23	C/T	0.43	0.31
BTNL2	rs2076530	6p21.3	A/G	29.78 *	0.26
CACNG4	rs4790896	17q24	A/G	0.36	0.41
CCR5	rs333	3p21	-/+	0.02	0.11
CD24	rs8734	6q21	C	NA	0.00 **
CNTF	rs1800169	11q12	A/G	0.80	0.12
CRYAB	rs14133	11q21-q23	C/G	0.08	0.27

Gene	rs-nr	Chromosome	Poly-morphism	HWE *	MAF
CRYAB	rs762550	11q21-q23	A/G	0.14	0.42
CRYAB	rs2234702	11q21-q23	C	NA	0.00 **
CTLA4	rs231775	2q33	A/G	1.03	0.37
CTLA4	rs5742909	2q33	C/T	0.45	0.09
EBF	rs1368297	5q34	A/T	0.06	0.38
GABBRA1	rs1805057	6p22	C	NA	0.00 **
HELZ	rs2363846	17q24	C/T	2.23	0.48
HLA	rs2395166	6p21.3	C/T	3.46	0.47
HLA	rs2213584	6p21.3	A/G	3.61	0.40
HLA	rs2227139	6p21.3	C/T	2.89	0.40
HLA	rs3135388	6p21.3	A/G	0.97	0.33
HLA	rs9268458	6p21.3	A/C	1.29	0.20
HLA	rs6457594	6p21.3	A/G	35.65 *	0.40
HLA-DRA	rs2395182	6p21.3	G/T	1.04	0.38
HLA-DRA	rs2239802	6p21.3	C/G	1.34	0.38
IFNAR1	rs2257167	21q22	C/G	0.00	0.08
IFNGR2	rs9808753	21q22	A/G	0.00	0.14
IKBL	rs3130062	6p21.3.	C/T	1.14	0.18
IL-10	rs1800896	1q32	A/G	0.56	0.46
IL1B	rs1799916	2q14	A	NA	0.00 **
IL1B	rs1143627	2q14	A/G	5.32 *	0.34
IL-1B	rs1143634	2q14	C/T	0.01	0.23
IL-1RN	rs419598	2q12-q14	C/T	0.53	0.31
IL-1RN	2073 Intron2 C/T (rs423904)	2q12-q14	C/T	0.72	0.30
IL-2	rs2069763	4q26	G/T	0.75	0.36
IL-2	rs2069762	4q26	G/T	0.31	0.27
IL-4R	rs1801275	16p12	A/G	0.37	0.20
IL7R	rs11567685	5p13	C/T	0.68	0.25
IL7R	rs7718919	5p13	G/T	0.22	0.13
IL7R	rs11567686	5p13	A/G	1.44	0.34
MC1R	rs1805009	16q24	C/G	0.02	0.01 **
MC1R	rs1805006	16q24	A/C	0.00	0.00 **
MEFV	rs28940577	16p13.3	A	NA	0.00 **
MGC33887	rs987931	17q24	G/T	0.39	0.32
MHC2TA	rs3087456	16p13	A/G	0.13	0.26
MOG	rs3130250	6p22	A/G	0.01	0.19
MOG	rs3130253	6p22	A/G	0.80	0.12
NDUFA7	rs2288414	19p13.2	C/G	7.90 *	0.03 **
NDUFA7	rs561	19p13.2	A/G	0.04	0.21
NDUFS5	rs2889683	1p34.2	C/T	2.63	0.31
NDUFS5	rs6981	1p34.2	A/G	105.96 *	0.04 **

Gene	rs-nr	Chromosome	Poly-morphism	HWE *	MAF
NDUFS7	rs2074897	19p13.3	A/G	6.21 *	0.47
NOS2A	rs1137933	17q11.2	A/G	0.49	0.25
NOS2A	rs2779248	17q11.2	C/T	0.00	0.39
NOTCH4	rs367398	6p21.3	A/G	0	0.16
PD-1	rs11568821	2q37	G/A	6.24 *	0.11
PITPNC1	rs1318	17q24	A/G	0.01	0.21
PITPNC1	rs2365403	17q24	C/G	0.55	0.18
PNMT	rs876493	17q11-q23	A/G	0.70	0.39
PRKCA	rs7220007	17q24	A/G	0.10	0.49
PRKCA	rs887797	17q24	C/T	0.50	0.30
PRKCA	rs2078153	17q24	C/G	0.91	0.23
PRKCA	rs3890137	17q24	A/G	0.44	0.37
PTPN22	rs2476601	1p13	A/G	2.29	0.11
PTPRC	rs17612648	1q31	C/G	0.11	0.03 **
PTPRC	rs4915154	1q31	A/G	0.00	0.00 **
CCL5	rs2280788	17q11.2-q12	C/G	0.06	0.02 **
CCL5	rs2107538	17q11.2-q12	C/T	0.00	0.18
Spp1	rs1126616	4q21	C/T	0.01	0.23
Spp1	rs1126772	4q21	A/G	0.23	0.18
Spp1	rs2853744	4q21	G/T	0.48	0.05
Spp1	rs9138	4q21	A/C	0.03	0.24
Spp1	rs4754	4q21	C/T	0.07	0.24
TNF-alpha	rs1800629	6p21.3	A/G	2.02	0.17
TRAIL	rs1131568	3q26	C/T	1.53	0.32
UCP2	rs659366	11q13	C/T	0.15	0.37
VDR	rs1544410	12q13	A/G	1.27	0.48
VDR	rs731236	12q13	A/G	0.39	0.48

* ChiSquare value. A value >3.84 indicates deviation from Hardy-Weinberg Equilibrium ($p<0.05$).

** Excluded due to minor allele frequency <0.05)

Table 6A: Additional SNPs included in the analyses

Gene	rs-nr
KIF1B	rs10492972
IGF2R	rs12202350
GRIN2A	rs8049651
KLC1	rs8702
IL7R	rs987107
STS	rs12861247
GPC6	rs7995215

Gene	rs-nr
EREG	rs1350666
ADRA1A	rs3808585
IL16	rs4128767
ARRB2	rs7208257
NTF3	rs7956189
IL12A	rs4680534
SLC6A4	rs1042173

Table 7: Examples of Probes Used in SNP Analysis

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
EBF1	Early B-cell Factor 1	rs1368297	intron 7 (271,440) A/T	TAAAGTTAGTC A GTTCTATGCTT
				TAAAGTTAGTC T GTTCTATGCTT
				AAGCATAGAAC T GACTAACTTTA
				AAGCATAGAAC A GACTAACTTTA
RANTES/CCL5	chemokine (C-C motif) ligand 5	rs2280788	-28C/G	GGGATGCCCCT C AACTGGCCCTA
				GGGATGCCCCT G AACTGGCCCTA
				TAGGGCCAGTT G AGGGGCATCCC
				TAGGGCCAGTT C AGGGGCATCCC
RANTES/CCL5	chemokine (C-C motif) ligand 5	rs2107538	-403G/A	AGGGAAAGGAG G TAAGATCTGTA
				AGGGAAAGGAG A TAAGATCTGTA
				TACAGATCTTA C CTCCTTTCCCT
				TACAGATCTTA T CTCCTTTCCCT
TGFB1	transforming growth factor, beta 1	rs17851976	L10P G869A	GTAGCAGCAGC G GCAGCAGCCGC
				GTAGCAGCAGC A GCAGCAGCCGC
				GCGGCTGCTGC C GCTGCTGCTAC
				GCGGCTGCTGC T GCTGCTGCTAC
UPC2	uncoupling protein 2	rs659366	–866G/A	GGGGTAACTGA C GCGTGAACAGC
				GGGGTAACTGA T GCGTGAACAGC
				GCTGTTCACGC G TCAGTTACCCC
				GCTGTTCACGC A TCAGTTACCCC
IKBL	inhibitory kappaB-like	rs3130062	C224R;738T/C	CAGAGGGATCC C GTCGACCCCCA
				CAGAGGGATCC T GTCGACCCCCA
				TGGGGGTCGAC G GGATCCCTCTG
				TGGGGGTCGAC A GGATCCCTCTG
Apo I/Fas (CD 95)	tumor necrosis factor receptor superfamily	rs1800682	–671A/G	GTCCATTCCAG A AACGTCTGTGA
				GTCCATTCCAG G AACGTCTGTGA
				TCACAGACGTT T CTGGAATGGAC
				TCACAGACGTT C CTGGAATGGAC
Apo I/Fas	tumor necrosis factor receptor	rs3781202	A/T (735)G/C intron	ATAAAATTTTC C TAGCAAATAAA

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
(CD 95)	superfamily		4	
				ATAAAATTTTC T TAGCAAATAAA
				TTTATTTGCTA G GAAAATTTTAT
				TTTATTTGCTA A GAAAATTTTAT
IL2	interleukin 2	rs2069763	114G/T	GAGCATTTACT G CTGGATTTACA
				GAGCATTTACT T CTGGATTTACA
				TGTAAATCCAG C AGTAAATGCTC
				TGTAAATCCAG A AGTAAATGCTC
IL2	interleukin 2	rs2069762	–385A/C	TTTTCTTTGTC A TAAAACTACAC
				TTTTCTTTGTC C TAAAACTACAC
				TTCAGTGTAGTTTTA T GACAAAGAAAATTTT
				TTCAGTGTAGTTTTA G GACAAAGAAAATTTT
IL10	interleukin 10	rs1800896	-1082G/A	GCTTCTTTGGGAAGGGGAAGTAGGG
				GCTTCTTTGGGAGGGGGAAGTAGGG
				CCCTACTTCCCCTTCCCAAAGAAGC
				CCCTACTTCCCCCTCCCAAAGAAGC
IL4R	interleukin 4 receptor	rs1801275	Q551R	CAGTGGCTATC G GGAGTTTGTAC
				CAGTGGCTATC A GGAGTTTGTAC
				TACAAACTCC C GATAGCCACT
				TACAAACTCC T GATAGCCACT
PTPRC	protein tyrosine phosphatase, receptor type, C	rs17612648	C77G	GCATTCTCACC C GCAAGCACCTT
				GCATTCTCACC G GCAAGCACCTT
				AAGGTGCTTGC G GGTGAGAATGC
				AAGGTGCTTGC C GGTGAGAATGC
PTPRC	protein tyrosine phosphatase, receptor type, C	rs4915154	A138G	TCACAGCGAAC G CCTCAGGTCTG
				TCACAGCGAAC A CCTCAGGTCTG
				CAGACCTGAGG C GTTCGCTGTGA
				CAGACCTGAGG T GTTCGCTGTGA
PD-1/PDCD1	programmed cell death 1	rs11568821	G7146A	AGCCCACCTGC G GTCTCCGGGGG
				AGCCCACCTGC A GTCTCCGGGGG
				CCCCCGGAGAC C GCAGGTGGGCT
				CCCCCGGAGAC T GCAGGTGGGCT
CRYAB	crystallin, alpha B	rs14133	-C249G	TGAAACAAGAC C ATGACAAGTCA
				TGAAACAAGAC G ATGACAAGTCA
				TGACTTGTCAT G GTCTTGTTTCA
				TGACTTGTCAT C GTCTTGTTTCA
CRYAB	crystallin, alpha B	rs762550	-A652G	GAGCCACATAGAACGAAAGATGC
				GAGCCACATAGGACGAAAGATGC
				GCATCTTTCGTTCTATGTGGCTC
				CATCTTTCGT C CTATGTGGCT

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
CRYAB	crystallin, alpha B	rs2234702	-C650G	GCCACATAGAA C GAAAGATGCAA
				GCCACATAGAA G GAAAGATGCAA
				TTGCATCTTTC G TTCTATGTGGC
				TTGCATCTTTC C TTCTATGTGGC
NDUFS5	NADH dehydrogenase (ubiquinone) Fe-S protein 5	rs2889683	-5649T/C	ACAACAGCAGA A ATAATAATCAA
				ACAACAGCAGA G ATAATAATCAA
				TTGATTATTAT T TCTGCTGTTGT
				TTGATTATTAT C TCTGCTGTTGT
NDUFS5	NADH dehydrogenase (ubiquinone) Fe-S protein 5	rs6981	3' UTR 5789 A/G	CAGCTGCTGAT A TCTGGAGGCTG
				CAGCTGCTGAT G TCTGGAGGCTG
				CAGCCTCCAGA T ATCAGCAGCTG
				CAGCCTCCAGA C ATCAGCAGCTG
NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7	rs2074897	intron 6 (6+71) A/G	GCCCTGATGGC A CTTATCAAAAG
				GCCCTGATGGC G CTTATCAAAAG
				CTTTTGATAAG T GCCATCAGGGC
				CTTTTGATAAG C GCCATCAGGGC
NDUFA7	NADH dehydrogenase (ubiquinone) 1 alpha	rs2288414	intron 2 (2+89) C/G	ATGTCAGCCCT C CGTTTCAGGGG
				ATGTCAGCCCT G CGTTTCAGGGG
				CCCCTGAAACG G AGGGCTGACAT
				CCCCTGAAACG C AGGGCTGACAT
NDUFA7	NADH dehydrogenase (ubiquinone) 1 alpha	rs561	9825 A/G	CCACCTCTTTAT A GGAGGAGCTGGA
				CCACCTCTTTAT G GGAGGAGCTGGA
				CCAGCTCCTCC T ATAAAGAGGTG
				CCAGCTCCTCC C ATAAAGAGGTG
ADAMTS14	ADAM metallopeptidase with thrombospondin type 1	rs4747075	intron 2 16860 A/G	CCCAGATGATG A CATTCGCCTTC
				CCCAGATGATG G CATTCGCCTTC
				GAAGGCGAATG T CATCATCTGGG
				GAAGGCGAATG C CATCATCTGGG
ADAMTS14	ADAM metallopeptidase with thrombospondin type 1	rs7081273	intron 2 24479 C/G	CATTTGGCAAA C GTAGGCTGGTC
				CATTTGGCAAA G GTAGGCTGGTC
				GACCAGCCTAC G TTTGCCAAATG
				GACCAGCCTAC C TTTGCCAAATG
ADAMTS14	ADAM metallopeptidase with thrombospondin type 1	rs4746060	intron 4 44225 C/T	GCACATCTATA C TGGGTCATCTT
				GCACATCTATA T TGGGTCATCTT
				AAGATGACCCA G TATAGATGTGC
				AAGATGACCCA A TATAGATGTGC
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	rs11569591	-708ins8	GCGTGGGGGGG T GGGGGCGAAGC
				GGGTGGGGGGG A GGGGGCGAAGC
				GCTTCGCCCCC A CCCCCCCACGC

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
				GCTTCGCCCCC T CCCCCCCACCC
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	rs11569591	-708ins8	CGTGGGGGGG T GGGGGCGAAG
				GGTGGGGGGG A GGGGGCGAAG
				CTTCGCCCCC A CCCCCCCACG
				CTTCGCCCCC T CCCCCCCACC
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	rs11569591	-708ins8	TGCGTGGGGGGG T GGGGGCGAAGCT
				GGGGTGGGGGGG A GGGGGCGAAGCT
				AGCTTCGCCCCC A CCCCCCCACGCA
				AGCTTCGCCCCC T CCCCCCCACCCC
SPP1	secreted phosphoprotein 1	rs28357094	-66[G/T]	GACACAATCTC G CCGCCTCCCTG
				GACACAATCTC T CCGCCTCCCTG
				CAGGGAGGCGG C GAGATTGTGTC
				CAGGGAGGCGG A GAGATTGTGTC
HLA-DR*1501	major histocompatibility complex, class II, DR	rs367398	-25 A/G (NOTCH4)	CTCCAAGCCCC A GTCCCTGTCCC
				CTCCAAGCCCC G GTCCCTGTCCC
				GGGACAGGGAC T GGGGCTTGGAG
				GGGACAGGGAC C GGGGCTTGGAG
HLA-DR*1501	major histocompatibility complex, class II, DR	rs1800629	-308G>A(TNF-alpha)	TGAGGGGCATG A GGACGGGGTTC
				TGAGGGGCATG G GGACGGGGTTC
				AACCCCGTCC T CATGCCCCTC
				AACCCCGTCC C CATGCCCCTC
IL7R	interleukin 7 receptor	rs11567685	-504T/C	GCATTTGCCTGCAGTCCTAGCTA
				GCATTTGCCTGTAGTCCTAGCTA
				TAGCTAGGACTGCAGGCAAATGC
				TAGCTAGGACTACAGGCAAATGC
IL7R	interleukin 7 receptor	rs7718919	-1085G/T	CACAAATGGGT G AGGCTGTATTC
				CACAAATGGGT T AGGCTGTATTC
				GAATACAGCCT C ACCCATTTGTG
				GAATACAGCCT A ACCCATTTGTG
IL7R	interleukin 7 receptor	rs11567686	-449A/G	CCTGGGAGGTG A AAATTGCAGTG
				CCTGGGAGGTG G AAATTGCAGTG
				CACTGCAATTT T CACCTCCCAGG
				CACTGCAATTT C CACCTCCCAGG
IFNAR1	interferon (alpha, beta and omega) receptor 1	rs2257167	V168L (G18417C)	ACATATAGCTTA C TTATCTGGAAAA
				ACATATAGCTTA G TTATCTGGAAAA
				TTTTCCAGATAA G TAAGCTATATGT
				TTTTCCAGATAA C TAAGCTATATGT
IFNAR2	interferon (alpha, beta and omega) receptor 2	rs7279064	F10V (11876T>G)	ATGCCTTCATC G TCAGATCACTT
				ATGCCTTCATC T TCAGATCACTT
				AAGTGATCTGA C GATGAAGGCAT

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
				AAGTGATCTGA A GATGAAGGCAT
IL1B	interleukin 1, beta proprotein	rs1799916	-511 A/C	AAGAGAATCCC A GAGCAGCCTGT
				AAGAGAATCCC C GAGCAGCCTGT
				ACAGGCTGCTC T GGGATTCTCTT
				ACAGGCTGCTC G GGGATTCTCTT
IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	rs9808753	Q64R	TGTTGTCTACC A AGTGCAGTTTA
				TGTTGTCTACC G AGTGCAGTTTA
				TAAACTGCACT T GGTAGACAACA
				TAAACTGCACT C GGTAGACAACA
Apo I/Fas (CD 95)	tumor necrosis factor receptor superfamily	rs2234978	E7(74) C>T	GAATCTCCAAC C TTAAATCCTGT
				GAATCTCCAAC T TTAAATCCTGT
				ACAGGATTTAA G GTTGGAGATTC
				ACAGGATTTAA A GTTGGAGATTC
CD24	CD24 antigen precursor	rs8734	V57A (226T>C)	CACCACCAAGG T GGCTGGTGGTG
				CACCACCAAGG C GGCTGGTGGTG
				CACCACCAGCC A CCTTGGTGGTG
				CACCACCAGCC G CCTTGGTGGTG
MEFV	Mediterranean fever protein	rs28940577	M694V	GGGTGGTGATA A TGATGAAGGAA
				GGGTGGTGATA G TGATGAAGGAA
				TTCCTTCATCA T TATCACCACCC
				TTCCTTCATCA C TATCACCACCC
CTLA4	cytotoxic T-lymphocyte-associated antigen 4	rs231775	+49A/G	TGAACCTGGCT A CCAGGACCTGG
				TGAACCTGGCT G CCAGGACCTGG
				CCAGGTCCTGG T AGCCAGGTTCA
				CCAGGTCCTGG C AGCCAGGTTCA
CNTF	ciliary neurotrophic factor	rs1800169	intron 1 (2-7) A/G	CCTGTATCCTC A GCCAGGTGAAG
				CCTGTATCCTC G GCCAGGTGAAG
				CTTCACCTGGC T GAGGATACAGG
				CTTCACCTGGC C GAGGATACAGG
MHC2TA	class II, major histocompatibility complex, transactivator	rs3087456	-168A/G	TTCAGAGGTGT A GGGAGGGCTTA
				TTCAGAGGTGT G GGGAGGGCTTA
				TAAGCCCTCCC T ACACCTCTGAA
				TAAGCCCTCCC C ACACCTCTGAA
VDR	vitamin D receptor	rs1544410	33062 A/G Intron	GACAGGCCTGC A CATTCCCAATA
				GACAGGCCTGC G CATTCCCAATA
				ATTGGGAATG T GCAGGCCTGT
				TTGGGAATG C GCAGGCCTG
PRKCA	protein kinase C, alpha	rs7220007	intron 3 264550 A/G	CCCCTGCTGGC A GATTGTTGCTA
				CCCCTGCTGGC G GATTGTTGCTA
				TAGCAACAATC T GCCAGCAGGGG
				TAGCAACAATC C GCCAGCAGGGG

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
PRKCA	protein kinase C, alpha	rs887797	intron 3 280475 C/T	GTCTTTTTAATA G CTGTAGACATCT
				GTCTTTTTAATA A CTGTAGACATCT
				GTCTTTTTAATA G CTGTAGACATCT
				GTCTTTTTAATA A CTGTAGACATCT
PRKCA	protein kinase C, alpha	rs2078153	intron 3 252845 C/G	AGTTACAGGGA C AAGAAGCCTTT
				AGTTACAGGGA G AAGAAGCCTTT
				AAAGGCTTCTT G TCCCTGTAACT
				AAAGGCTTCTT C TCCCTGTAACT
CTLA4	cytotoxic T-lymphocyte-associated protein 4	rs5742909	-318C/T	ATCCAGATCCT C AAAGTGAACAT
				ATCCAGATCCT T AAAGTGAACAT
				ATGTTCACTTT G AGGATCTGGAT
				ATGTTCACTTT A AGGATCTGGAT
MGC33887	coiled-coil domain containing 46	rs987931	intron 21 413506 G/T	GCAGCAGTTT G CCCTGTGAGT
				GCAGCAGTTT T CCCTGTGAGT
				ACTCACAGGG C AAACTGCTGC
				ACTCACAGGG A AAACTGCTGC
CACNG4	calcium channel, voltage-dependent, gamma subunit 4	rs4790896	intron 1 15546 C/T	GACTCCGATGA A GTTTGAGCAGA
				GACTCCGATGA G GTTTGAGCAGA
				TCTGCTCAAAC T TCATCGGAGTC
				TCTGCTCAAAC C TCATCGGAGTC
HELZ	helicase with zinc finger	rs2363846	intron 18 68091 C/T	TCAATAATAAA C ATCATCTGACC
				TCAATAATAAA T ATCATCTGACC
				GGTCAGATGAT G TTTATTATTGA
				GGTCAGATGAT A TTTATTATTGA
PITPNC1	phosphatidylinositol transfer protein, cytoplasmic 1	rs1318	C/T	TGGGTGGTGTA A ATATTCCTTTA
				TGGGTGGTGTA G ATATTCCTTTA
				GCTAAAGGAATAT T TACACCACCCACC
				GCTAAAGGAATAT C TACACCACCCACC
PITPNC1	phosphatidylinositol transfer protein, cytoplasmic 1	rs2365403	C/G	ACTGACTTTCT C TGCCTAATGTA
				ACTGACTTTCT G TGCCTAATGTA
				TACATTAGGCA G AGAAAGTCAGT
				TACATTAGGCA C AGAAAGTCAGT
MC1R	melanocortin 1 receptor	rs1805009	294 D/H	ATGCCATCATC C ACCCCCTCATC
				ATGCCATCATC G ACCCCCTCATC
				GATGAGGGGGT G GATGATGGCAT
				GATGAGGGGGT C GATGATGGCAT
MC1R	melanocortin 1 receptor	rs1805006	84 Asp/Glu	GCCTTGTCGGA A CTGCTGGTGAG
				GCCTTGTCGGA C CTGCTGGTGAG
				CTCACCAGCAG T TCCGACAAGGC
				CTCACCAGCAG G TCCGACAAGGC

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
PRKCA	protein kinase C, alpha	rs1010544	intron 8 388476 C/T	TAAAAAGGTGC A TGTATCTGTGT
				TAAAAAGGTGC G TGTATCTGTGT
				ACACAGATACA T GCACCTTTTTA
				ACACAGATACA C GCACCTTTTTA
PRKCA	protein kinase C, alpha	rs3890137	intron 8 427857 A/G	GGCTGGCTTT A CCACAGACTG
				TGGCTGGCTTT G CCACAGACTGT
				CAGTCTGTGG T AAAGCCAGCC
				ACAGTCTGTGG C AAAGCCAGCCA
BTNL2 (DRb1*15)	butyrophilin-like 2	rs2076530	11084C/T	TGAAGGTGGTA A GTAAGAATTCT
				TGAAGGTGGTA G GTAAGAATTCT
				AGAATTCTTAC T TACCACCTTCA
				AGAATTCTTAC C TACCACCTTCA
PNMT	phenylethanolamine N-methyltransferase	rs876493	-184G/A	CACTCACCTCC A GTGTGTCTGCA
				CACTCACCTCC G GTGTGTCTGCA
				CACTCACCTCC A GTGTGTCTGCA
				CACTCACCTCC G GTGTGTCTGCA
PNMT	phenylethanolamine N-methyltransferase	rs3764351	-390G/A	ATGGCTGCGGG A GGCTGGAGAAG
				ATGGCTGCGGG G GGCTGGAGAAG
				CTTCTCCAGCC T CCCGCAGCCAT
				CTTCTCCAGCC C CCCGCAGCCAT
TRAIL/TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	rs9880164 (rs1131568 in v. 37.1)	1595C/T	GCTAATTTTTG C ACTTTCAGTAG
				GCTAATTTTTG T ACTTTCAGTAG
				CTACTGAAAGT G CAAAAATTAGC
				CTACTGAAAGT A CAAAAATTAGC
PTPN22	protein tyrosine phosphatase, non-receptor type 22	rs2476601	1858C/T: (620 W/R)	TTCAGGTGTCC A TACAGGAAGTG
				TTCAGGTGTCC G TACAGGAAGTG
				CACTTCCTGTA T GGACACCTGAA
				CACTTCCTGTA C GGACACCTGAA
MOG	myelin oligodendrocyte glycoprotein	rs3130250	15G/A [S5S]	GCAAGCTTATC A AGACCCTCTCT
				GCAAGCTTATC G AGACCCTCTCT
				AGAGAGGGTCT T GATAAGCTTGC
				AGAGAGGGTCT C GATAAGCTTGC
MOG	myelin oligodendrocyte glycoprotein	rs3130253	520G/A [V145I]	CTGTTGGCCTC A TCTTCCTCTGC
				CTGTTGGCCTC G TCTTCCTCTGC
				GCAGAGGAAGA T GAGGCCAACAG
				GCAGAGGAAGA C GAGGCCAACAG
SPP1	secreted phosphoprotein 1	rs9138	1286 A/C	ATTTATGTAGA A GCAAACAAAAT
				ATTTATGTAGA C GCAAACAAAAT
				ATTTTGTTTGC T TCTACATAAAT
				ATTTTGTTTGC G TCTACATAAAT

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
SPP1	secreted phosphoprotein 1	rs4754	282T/C	GAAGATGATGA C GACCATGTGGA
				GAAGATGATGA T GACCATGTGGA
				TCCACATGGTC G TCATCATCTTC
				TCCACATGGTC A TCATCATCTTC
SPP1	secreted phosphoprotein 1	rs1126616	750C/T	AAGCGGAAAGC C AATGATGAGAG
				AAGCGGAAAGC T AATGATGAGAG
				CTCATCATT G GCTTTCCGC
				CTCATCATT A GCTTTCCGC
SPP1	secreted phosphoprotein 1	rs1126772	1083A/G	TGGAAATAACT A ATGTGTTTGAT
				TGGAAATAACT G ATGTGTTTGAT
				ATCAAACACAT T AGTTATTTCCA
				ATCAAACACAT C AGTTATTTCCA
HLA-DRA	major histocompatibility complex, class II, DR alpha	rs2395182	G/T	AGATGCCTATT G TATTACCGAGA
				AGATGCCTATT T TATTACCGAGA
				TCTCGGTAATA C AATAGGCATCT
				TCTCGGTAATA A AATAGGCATCT
HLA	major histocompatibility complex	rs2395166	C/T	ATAAGGTGAAA C AGAAACAGATC
				ATAAGGTGAAA T AGAAACAGATC
				GATCTGTTTCT G TTTCACCTTAT
				GATCTGTTTCT A TTTCACCTTAT
HLA	major histocompatibility complex	rs2213584	A/G	TGAGCAAAGAG A TTGGACACTGA
				TGAGCAAAGAG G TTGGACACTGA
				TCAGTGTCCAA T CTCTTTGCTCA
				TCAGTGTCCAA C CTCTTTGCTCA
HLA	major histocompatibility complex	rs2227139	C/T	CAACAGTTCAT C GTGTTTCAAAT
				CAACAGTTCAT T GTGTTTCAAAT
				ATATTTGAAACTC G ATGAACTGTTGCT
				ATATTTGAAACTC A ATGAACTGTTGCT
IL1RN	interleukin 1 receptor antagonist	rs419598	2018 T/C	CCAACTAGTTGCTGGATACTTGCAA
				CCAACTAGTTGCCGGATACTTGCAA
				TTGCAAGTATCCAGCAACTAGTTGG
				TTGCAAGTATCCGGCAACTAGTTGG
IL1RN	interleukin 1 receptor antagonist	2073 Intron2 C/T (rs423904)	2073 C/T Intron2	TGCCAGGAAAG C CAATGTATGTG
				TTGCCAGGAAAG T CAATGTATGTGG
				CCACATACATTG G CTTTCCTGGCAA
				CCACATACATTG A CTTTCCTGGCAA
NOS2A	nitric oxide synthase 2A isoform 1	rs1137933	exon 10 C/T, D346D	TAGCGCTGGAC A TCACAGAAGTC
				TAGCGCTGGAC G TCACAGAAGTC
				GACTTCTGTGA T GTCCAGCGCTA
				GACTTCTGTGA C GTCCAGCGCTA
GABBRA1	gamma-aminobutyric acid (GABA) B receptor 1	rs1805057	G1465A (489 G/S)	ACCAGAACGGC C GCCTCCTCCAG

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
				ACCAGAACGGC T GCCTCCTCCAG
				CTGGAGGAGGC G GCCGTTCTGGT
				CTGGAGGAGGC A GCCGTTCTGGT
VDR	vitamin D receptor	rs731236	Taq 1	TGGATGGCCTC A ATCAGCGCGGC
				TGGATGGCCTC G ATCAGCGCGGC
				GCCGCGCTGAT T GAGGCCATCCA
				GCCGCGCTGAT C GAGGCCATCCA
NOS2A	nitric oxide synthase 2A isoform 1	rs2779248	-277 A/G	GGCTGCTAAGA C AGAGGCACCAC
				GGCTGCTAAGA T AGAGGCACCAC
				GTGGTGCCTCT G TCTTAGCAGCC
				GTGGTGCCTCT A TCTTAGCAGCC
IL1B	interleukin 1, beta	rs1143627	-31 Tata	CTTTTGAAAGC T ATAAAAACAGC
				CTTTTGAAAGC C ATAAAAACAGC
				CTTTTGAAAGC T ATAAAAACAGC
				CTTTTGAAAGC C ATAAAAACAGC
HLA-DRA	major histocompatibility complex, class II, DR alpha	rs2239802	intron 4 4118 C/G	CCAGATGATAC C AATGTCTGATT
				CCAGATGATAC G AATGTCTGATT
				AATCAGACATT G GTATCATCTGG
				AATCAGACATT C GTATCATCTGG
IL1B	interleukin 1, beta	rs1143634	+3953-4	CCTATCTTCTT C GACACATGGGA
				CCTATCTTCTT T GACACATGGGA
				TCCCATGTGTC G AAGAAGATAGG
				TCCCATGTGTC A AAGAAGATAGG
SPP1	secreted phosphoprotein 1	rs2853744	-616G/T	GCAGTCATCCT G CTCTCAGTCAG
				GCAGTCATCCT T CTCTCAGTCAG
				CTGACTGAGAG C AGGATGACTGC
				CTGACTGAGAG A AGGATGACTGC
CCR5	chemokine (C-C motif) receptor 5	rs333	CCR5*D32	TTTTCCATACAGTCAGTATCAAT
				TTTTCCATACATTAAAGATAGTC
				ATTGATACTGACTGTATGGAAAA
				GACTATCTTTAATGTATGGAAAA
HLA-DRA	major histocompatibility complex, class II, DR alpha	rs3135388	3' UTR 5323 C/T	CCTAAAGTGGG A TTGGTTTGTTG
				CCTAAAGTGGG G TTGGTTTGTTG
				CAACAAACCAA T CCCACTTTAGG
				CAACAAACCAA C CCCACTTTAGG
HLA	major histocompatibility complex	rs9268458	A/C	AAAGTGCTCGG A TGTTGGGATTA
				AAAGTGCTCGG C TGTTGGGATTA
				TAATCCCAACA T CCGAGCACTTT
				TAATCCCAACA G CCGAGCACTTT
HLA	major histocompatibility complex	rs6457594	A/G	TCCACACATAC A GGTTTGTCACT
				TCCACACATAC G GGTTTGTCACT

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
				AGTGACAAACC T GTATGTGTGGA
				AGTGACAAACC C GTATGTGTGGA
HLA	major histocompatibility complex	rs7451962	A/G	GGCAGGAATTC A GAATCCCTCAT
				GGCAGGAATTC G GAATCCCTCAT
				ATGAGGGATTC T GAATTCCTGCC
				ATGAGGGATTC C GAATTCCTGCC
HLA	major histocompatibility complex	rs7451962	A/G	GGGCAGGAATTC A GAATCCCTCATC
				GGGCAGGAATTC G GAATCCCTCATC
				GATGAGGGATTC T GAATTCCTGCCC
				GATGAGGGATTC C GAATTCCTGCCC
HLA	major histocompatibility complex	rs7451962	A/G	GCAGGAATTC A GAATCCCTCA
				GCAGGAATTC G GAATCCCTCA
				TGAGGGATTC T GAATTCCTGC
				TGAGGGATTC C GAATTCCTGC
PNMT	phenylethanolamine N-methyltransferase	rs3764351	-390G/A	ATGGCTGCGGG A GGCTGGAGAAG
				ATGGCTGCGGG G GGCTGGAGAAG
				TTCTCCAGCC T CCCGCAGCCA
				TTCTCCAGCC C CCCGCAGCCA
KIF1B	kinesin family member 1B	rs10492972	C/T	CGCTACAATTCT C CTGGTCAGGTTT
				CGCTACAATTCT T CTGGTCAGGTTT
				AAACCTGACCAG G AGAATTGTAGCG
				AAACCTGACCAG A AGAATTGTAGCG
IGF2R	Immunoglobulin G Fc Receptor II	rs12202350	C/T	GATAACTTCACA C AGATTGAAATGT
				GATAACTTCACA T AGATTGAAATGT
				ACATTTCAATCT G TGTGAAGTTATC
				ACATTTCAATCT A TGTGAAGTTATC
GRIN2A	glutamate receptor, ionotropic, N-methyl D-aspartate 2A	rs8049651	C/T	ACACGTCTCGGT C AGGGGGTCTATG
				ACACGTCTCGGT T AGGGGGTCTATG
				CATAGACCCCCT G ACCGAGACGTGT
				CATAGACCCCCT A ACCGAGACGTGT
KLC1	kinesin light chain 1	rs8702	C/G	ACATGCCTTGCT C TAAGGCTTAGTT
				ACATGCCTTGCT G TAAGGCTTAGTT
				AACTAAGCCTTA G AGCAAGGCATGT
				AACTAAGCCTTA C AGCAAGGCATGT
IL7R	interleukin 7 receptor	rs987107	C/T	TCTCTTTACTGA C AGCAACTCTGGC
				TCTCTTTACTGA T AGCAACTCTGGC
				GCCAGAGTTGCT G TCAGTAAAGAGA
				GCCAGAGTTGCT A TCAGTAAAGAGA
STS	STS steroid sulfatase, isozyme S	rs12861247	A/G	CAGGGAGGAATG A ACCTGGATTCCT
				CAGGGAGGAATG G ACCTGGATTCCT
				AGGAATCCAGGT T CATTCCTCCCTG

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
				AGGAATCCAGGT C CATTCCTCCCTG
GPC6	glypican 6	rs7995215	A/G	TGCACACTTCAG A ATGTTTGGCACC
				TGCACACTTCAG G ATGTTTGGCACC
				GGTGCCAAACAT T CTGAAGTGTGCA
				GGTGCCAAACAT C CTGAAGTGTGCA
EREG	epiregulin	rs1350666	C/T	TGGCTATTGTTT C ATTGCATTCACT
				TGGCTATTGTTT T ATTGCATTCACT
				AGTGAATGCAAT G AAACAATAGCCA
				AGTGAATGCAAT A AAACAATAGCCA
ADRA1A	adrenergic, alpha-1A-, receptor	rs3808585	C/T	GGGGTAGAGGGG C CGGTATAAAACC
				GGGGTAGAGGGG T CGGTATAAAACC
				GGTTTTATACCG G CCCCTCTACCCC
				GGTTTTATACCG A CCCCTCTACCCC
IL16	interleukin 16	rs4128767	C/T	GCTGTACCATAG C TTTTCTGAGAAA
				GCTGTACCATAG T TTTTCTGAGAAA
				TTTCTCAGAAAA G CTATGGTACAGC
				TTTCTCAGAAAA A CTATGGTACAGC
ARRB2	arrestin, beta 2	rs7208257	C/T	TGAAGTCTTCTC C TTCCTCCGCCAC
				TGAAGTCTTCTC T TTCCTCCGCCAC
				GTGGCGGAGGAA G GAGAAGACTTCA
				GTGGCGGAGGAA A GAGAAGACTTCA
NTF3	neurotrophin-3	rs7956189	A/G	TAAGTAAGTGGC A GAGTGAAGATTG
				TAAGTAAGTGGC G GAGTGAAGATTG
				CAATCTTCACTC T GCCACTTACTTA
				CAATCTTCACTC C GCCACTTACTTA
IL12A	interleukin-12 subunit alpha	rs4680534	C/T	ATCTATGTGTGT C TGTACATGAATA
				ATCTATGTGTGT T TGTACATGAATA
				TATTCATGTACA G ACACACATAGAT
				TATTCATGTACA A ACACACATAGAT
SLC6A4	solute carrier family 6 , member 4	rs1042173	G/T	GAGTAGCATATA G AATTTTATTGCT
				GAGTAGCATATA T AATTTTATTGCT
				AGCAATAAAATT C TATATGCTACTC
				AGCAATAAAATT A TATATGCTACTC
FLJ34870	FLJ34870	rs7577925	A/G	TCCTTGACTGTT A GACACCAAGGAG
				TCCTTGACTGTT G GACACCAAGGAG
				CTCCTTGGTGTC T AACAGTCAAGGA
				CTCCTTGGTGTC C AACAGTCAAGGA
FCRL3	Fc receptor-like 3	rs7528684	nearGene-5' A/G	ATGTACAGATCA A GGACTTCCCGTA
				ATGTACAGATCA G GGACTTCCCGTA
				TACGGGAAGTCC T TGATCTGTACAT
				TACGGGAAGTCC C TGATCTGTACAT

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
IGF2R	insulin-like growth factor 2 receptor	rs6917747	A/G	CTGGGAGAGACT A GCTCACACAGCT
				CTGGGAGAGACT G GCTCACACAGCT
				AGCTGTGTGAGC T AGTCTCTCCCAG
				AGCTGTGTGAGC C AGTCTCTCCCAG
LOC729293	LOC729293	rs6570426	A/T	CCCTTCCAAATA A CCAATCATACAC
				CCCTTCCAAATA T CCAATCATACAC
				GTGTATGATTGG T TATTTGGAAGGG
				GTGTATGATTGG A TATTTGGAAGGG
SNAP25	synaptosomal-associated protein, 25kDa	rs6077690	A/T	CACTTTGGAAAA A ATTCTGACTACA
				CACTTTGGAAAA T ATTCTGACTACA
				TGTAGTCAGAAT T TTTTCCAAAGTG
				TGTAGTCAGAAT A TTTTCCAAAGTG
MORF4	mortality factor 4	rs4473631	A/C	CAGAGGACAATT A TCTTGGAAAGCA
				CAGAGGACAATT C TCTTGGAAAGCA
				TGCTTTCCAAGA T AATTGTCCTCTG
				TGCTTTCCAAGA G AATTGTCCTCTG
SNAP25	synaptosomal-associated protein, 25kDa	rs3787283	C/T	AATTCCAGAAAA C GAATGATTCCCA
				AATTCCAGAAAA T GAATGATTCCCA
				TGGGAATCATTC G TTTTCTGGAATT
				TGGGAATCATTC A TTTTCTGGAATT
LOC728594	hypothetical protein LOC728594	rs3756450	C/T	CCACAATGATAA C AAAGCCGACTTG
				CCACAATGATAA T AAAGCCGACTTG
				CAAGTCGGCTTT G TTATCATTGTGG
				CAAGTCGGCTTT A TTATCATTGTGG
SLC6A2	solute carrier family 6 member 2	rs28386840	A/T	GGGCTGAGCACC A GTTTCCCCAGCA
				GGGCTGAGCACC T GTTTCCCCAGCA
				TGCTGGGGAAAC T GGTGCTCAGCCC
				TGCTGGGGAAAC A GGTGCTCAGCCC
ZNF544	zinc finger protein 544	rs260461	A/G	ATCAATGTCACT A GATCAAAATCAA
				ATCAATGTCACT G GATCAAAATCAA
				TTGATTTTGATC T AGTGACATTGAT
				TTGATTTTGATC C AGTGACATTGAT
MHC II/HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	rs2187668	A/G	AGCTGAGAGTAA G TGAGGACCATGT
				AGCTGAGAGTAA A TGAGGACCATGT
				ACATGGTCCTCA C TTACTCTCAGCT
				ACATGGTCCTCA T TTACTCTCAGCT
SLC6A4	solute carrier family 6, member 4	rs2066713	C/T	GCATTTCCCTTC C GTAGACCCTCTG
				GCATTTCCCTTC T GTAGACCCTCTG
				CAGAGGGTCTAC G GAAGGGAAATGC
				CAGAGGGTCTAC A GAAGGGAAATGC
CSMD1	CUB and Sushi multiple domains 1	rs2049306	A/C	GTTCTGAAAGCA A ACATTTAAATAT

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
				GTTCTGAAAGCA C ACATTTAAATAT
				ATATTTAAATGT T TGCTTTCAGAAC
				ATATTTAAATGT G TGCTTTCAGAAC
SLC1A3	solute carrier family 1 member 3	rs2032893	A/G	ATAAATAAATAT A CAGAAGCATTGG
				ATAAATAAATAT G CAGAAGCATTGG
				CCAATGCTTCTG T ATATTTATTTAT
				CCAATGCTTCTG C ATATTTATTTAT
LOC647094	LOC647094	rs2028455	A/G	ACATGCCTGCCT A GAATGATTACTT
				ACATGCCTGCCT G GAATGATTACTT
				AAGTAATCATTC T AGGCAGGCATGT
				AAGTAATCATTC C AGGCAGGCATGT
DMRT2	doublesex and mab-3 related transcription factor 2	rs17641078	C/G	AAGATCAGCAAA C AAAACACCAGGC
				AAGATCAGCAAA G AAAACACCAGGC
				GCCTGGTGTTTT G TTTGCTGATCTT
				GCCTGGTGTTTT C TTTGCTGATCTT
DBH	dopamine beta-hydroxylase (dopamine beta-monooxygenase)	rs1611115	C/T	TCAGTCTACTTG C GGGAGAGGACAG
				TCAGTCTACTTG T GGGAGAGGACAG
				CTGTCCTCTCCC G CAAGTAGACTGA
				CTGTCCTCTCCC A CAAGTAGACTGA
MMP24	MMP24 matrix metallopeptidase 24	rs1555322	A/G	CACGCACTTCAC A TGTATCTTATTC
				CACGCACTTCAC G TGTATCTTATTC
				GAATAAGATACA T GTGAAGTGCGTG
				GAATAAGATACA C GTGAAGTGCGTG
DSEL	DSEL	rs13353224	A/G	ATCAGAGTTAAT A AACTTCCCTATT
				ATCAGAGTTAAT G AACTTCCCTATT
				AATAGGGAAGTT T ATTAACTCTGAT
				AATAGGGAAGTT C ATTAACTCTGAT
C1orf125	chromosome 1 open reading frame 125	rs12047808	A/G	AATGAGAGGGGT A ACACACATTATG
				AATGAGAGGGGT G ACACACATTATG
				CATAATGTGTGT T ACCCCTCTCATT
				CATAATGTGTGT C ACCCCTCTCATT
GPC5	glypican 5	rs10492503	A/T	TGGATAACTGCT A CAATTATAGTTT
				TGGATAACTGCT T CAATTATAGTTT
				AAACTATAATTG T AGCAGTTATCCA
				AAACTATAATTG A AGCAGTTATCCA
C1GALT1	core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1	rs10259085	C/T	TAAAAACAATTA C GTAACACCAAGA
				TAAAAACAATTA T GTAACACCAAGA
				TCTTGGTGTTAC G TAATTGTTTTTA
				TCTTGGTGTTAC A TAATTGTTTTTA
MET	met proto-oncogene (hepatocyte growth	rs10243024	A/G	TATTTTTACTCC A AATACTGTTTCA

Gene Symbol	Gene Name	rs ID	SNP	Oligonucleotide sequence (5' > 3')
	factor receptor)			
				TATTTTTACTCC G AATACTGTTTCA
				TGAAACAGTATT T GGAGTAAAAATA
				TGAAACAGTATT C GGAGTAAAAATA
ICOS	inducible T-cell co-stimulator	rs4404254	C/T	TTACAAGTTTAG C TCTTTTTGTAGA
				TTACAAGTTTAG T TCTTTTTGTAGA
				TCTACAAAAAGA G CTAAACTTGTAA
				TCTACAAAAAGA A CTAAACTTGTAA
OAS1	2',5'-oligoadenylate synthetase 1	rs3741981/rs11314 54	A/G	CAGTTGACTGGC A GCTATAAACCTA
				CAGTTGACTGGC G GCTATAAACCTA
				TAGGTTTATAGC T GCCAGTCAACTG
				TAGGTTTATAGC C GCCAGTCAACTG

Table 8: Examples of Forward Primers Used in SNP Analysis

SNP#	Gene Symbol	rs ID	Forward Primers (sequence 5' >3')
1	EBF1	rs1368297	CCAAATCTTGGTTTTCAGTGC
2	RANTES/CCL5	rs2280788	TATGATACCGGCCAATGCTT
3	RANTES/CCL5	rs2107538	CACCTCCTTTGGGGACTGTA
4	TGFB1	rs17851976	TCGATAGTCTTGCAGGTGGA
6	UPC2	rs659366	TTCGCCTTTAATTGGCTGAC
7	IKBL	rs3130062	TGAGTCCTTCTCAGCCTGGT
8	Apo I/Fas (CD 95)	rs1800682	CCTATGGCGCAACATCTGTA
9	Apo I/Fas (CD 95)	rs3781202	CCAATGCCTACCTAGCCTGT
10	IL2	rs2069763	GCATTGCACTAAGTCTTGCAC
11	IL2	rs2069762	ACCCCCAAAGACTGACTGAA
12	IL10	rs1800896	ATGGAGGCTGGATAGGAGGT
13	IL4R	rs1801275	CAACCTGAGCCAGAAACCTG
14	PTPRC	rs17612648	ATGCCCAGTGTTCCACTTTC
15	PTPRC	rs4915154	GCAGATGTCCCAGGAGAGAG
16	PD-1/PDCD1	rs11568821	TATAGCCAGGACCCCACCTC
17	CRYAB	rs14133	TGCTTGGGATTCCTGACTCT
18	CRYAB	rs762550	GCACCCAATTCCTAAAGCAC
19	CRYAB	rs2234702	GCACCCAATTCCTAAAGCAC
20	NDUFS5	rs2889683	TTGCTCAACTTTAGTTTTTCAGTCA
21	NDUFS5	rs6981	GCAGCGGGATAAGCTGATAA
22	NDUFS7	rs2074897	GGTCTCCAGGGACAGACGTA
24	NDUFA7	rs2288414	CGCTGAGCACTGCAAATCTA
25	NDUFA7	rs561	CCAAGGAGGCAAAGTAGTCG
26	ADAMTS14	rs4747075	TCCATTGTGGGGATTTTTGT

SNP#	Gene Symbol	rs ID	Forward Primers (sequence 5' >3')
27	ADAMTS14	rs7081273	GCCTTGGAAGGAGAAAGGAG
28	ADAMTS14	rs4746060	CTGGGGAGGTGCTATGGAT
29	NFKBIA	rs11569591	AGGCTTTTCACTCCTCCAAA
29	NFKBIA	rs11569591	AGGCTTTTCACTCCTCCAAA
29	NFKBIA	rs11569591	AGGCTTTTCACTCCTCCAAA
30	SPP1	rs28357094	TGTGTGTGTGCGTTTTTGTTT
31	HLA-DR*1501	rs367398	TGAGACACATAGCAGCAGCA
32	HLA-DR*1501	rs1800629	GCCCCTCCCAGTTCTAGTTC
34	IL7R	rs11567685	GCAGGCAGATCACTTGAGGT
35	IL7R	rs7718919	GCTCTGCCATTGTTGCATAA
36	IL7R	rs11567686	CCGTCTCCACTGAAAACACA
37	IFNAR1	rs2257167	GCTCAGATTGGTCCTCCAGA
38	IFNAR2	rs7279064	TCTTGTCTTTGCTCCCATTTTT
39	IL1B	rs1799916	GGCAGAGAGACAGAGAGACTCC
40	IFNGR2	rs9808753	TGTACAACGCAGAGCAGGTC
41	Apo I/Fas (CD 95)	rs2234978	TGCAGAAAGCACAGAAAGGA
42	CD24	rs8734	ACCCACGCAGATTTATTCCA
43	MEFV	rs28940577	TTGGAGACAAGACAGCATGG
44	CTLA4	rs231775	GGATCCTGAAAGGTTTTGCTC
45	CNTF	rs1800169	GACACTGGGGTGATGACAGA
46	MHC2TA	rs3087456	AGGTTCCCCCAACAGACTTT
47	VDR	rs1544410	CCTCACTGCCCTTAGCTCTG
48	PRKCA	rs7220007	AGCTGAGTGTTGTGCAGTGG
49	PRKCA	rs887797	AACCCCTGCATTTCAGAATTT
50	PRKCA	rs2078153	AAACAACTCCACCCAGGTTC
51	CTLA4	rs5742909	TGGTTAAGGATGCCCAGAAG
52	MGC33887	rs987931	CTTCGATAAATAGTGCTGGGAAA
53	CACNG4	rs4790896	CTTAATCGGAAAGCTGTGTCG
54	HELZ	rs2363846	GGAAAACACCAACACTCTCCA
55	PITPNC1	rs1318	TCAGTTGCAAAGCTACGATGA
56	PITPNC1	rs2365403	ACGCCTTTGGAACAACAATC
57	MC1R	rs1805009	AACCTCTTTCTCGCCCTCAT
58	MC1R	rs1805006	TGCACTCACCCATGTACTGC
59	PRKCA	rs1010544	ACCAGCTTGCAGTCTCTGCT
60	PRKCA	rs3890137	AGCCAGGAGACCTGAGACTG
61	BTNL2 (DRb1*15)	rs2076530	TACTCAGTGCCAGACCTTCG
62	PNMT	rs876493	TAAAGATTGTGGGGGTGAGG
63	PNMT	rs3764351	AAAGGGCCTAATTCCCCAGT
64	TRAIL/TNFSF10	rs9880164 (rs1131568 in v. 37.1)	ACTACAGGCATGTGCCAACA
65	PTPN22	rs2476601	TGCCCATCCCACACTTTATT
66	MOG	rs3130250	TCTGTCCCCAGGAACAGTAGA
67	MOG	rs3130253	ATGCTGAGTGTTGGGGATTC

SNP#	Gene Symbol	rs ID	Forward Primers (sequence 5' >3')
68	SPP1	rs9138	GCTTCATGGAAACTCCCTGT
69	SPP1	rs4754	AGACCCTTCCAAGTAAGTCCAA
70	SPP1	rs1126616	AGAGTGCTGAAACCCACAGC
71	SPP1	rs1126772	GAACATGAAATGCTTCTTTCTCAG
72	HLA-DRA	rs2395182	GACTGGCCTTACCCATTCTG
73	HLA	rs2395166	CGCTTTCCATAGAAACCTTGG
74	HLA	rs2213584	CATTGCAGGATTTACATATCAACA
75	HLA	rs2227139	CAGCCAAGATGAAACCCAAG
76	IL1RN	rs419598	ACAAGTTCTGGGGGACACAG
77	IL1RN	2073 Intron2 C/T (rs423904)	ACAAGTTCTGGGGGACACAG
78	NOS2A	rs1137933	CAGAGTGATAGCGGCGAGT
79	GABBRA1	rs1805057	TGGTCGGTAATGGTCTGGTT
80	VDR	rs731236	AGGTCGGCTAGCTTCTGGAT
81	NOS2A	rs2779248	CTCTGTGTGGTGCCTCTTCA
82	IL1B	rs1143627	CAGTTTCTCCCTCGCTGTTT
83	HLA-DRA	rs2239802	TGATCAAGGTGCCCGTCTAT
84	IL1B	rs1143634	ATGCTCAGGTGTCCTCCAAG
85	SPP1	rs2853744	ACACAGCGGAATTCAGAACC
87	CCR5	rs333	CGTCTCTCCCAGGAATCATC
88	HLA-DRA	rs3135388	CATTTGGGCTTGGTCTCATT
89	HLA	rs9268458	AATGGGGCCTCACTATGTTG
90	HLA	rs6457594	TGAATTCTGGGGGCTTACTG
91	HLA	rs7451962	GCCAGCTCAGTGAGGTCAGTA
92	HLA	rs7451962	GCCAGCTCAGTGAGGTCAGTA
93	HLA	rs7451962	GCCAGCTCAGTGAGGTCAGTA
94	PNMT	rs3764351	AAAGGGCCTAATTCCCCAGT
95	KIF1B	rs10492972	TGACCTCACATTGGCTATTGG
96	IGF2R	rs12202350	ATAGGCATAAGCCACCATGC
97	GRIN2A	rs8049651	AGCATTCCTGCCACTCACTT
98	KLC1	rs8702	AGAAAAGCAGAATGCCCAAA
99	IL7R	rs987107	ACCTCTGGGAAAAAGCCCTA
100	STS	rs12861247	TAAACAAGGAAGGGCACTGG
101	GPC6	rs7995215	CAGCAGTGTCCATGAGAATCA
102	EREG	rs1350666	TTGGGGGCTATTTAAGTTCA
103	ADRA1A	rs3808585	CTCGGGCAAAGACTCTTGTT
104	IL16	rs4128767	ATGATCACACCACTGCATCC
105	ARRB2	rs7208257	CAGCGTCTCCAGCCTCTTAG
106	NTF3	rs7956189	AATCCTTTGAGGGAGCCAGT
107	IL12A	rs4680534	TCAGGTTTTCCTCCTACTTCAAA
108	SLC6A4	rs1042173	AAACTGCGTAGGAGAGAACAGG

SNP#	Gene Symbol	rs ID	Forward Primers (sequence 5' >3')
109	FLJ34870	rs7577925	TGGGAGCAAAGTGAAAGTCA
110	FCRL3	rs7528684	TCACACAGCCTTTGGTTCTG
111	IGF2R	rs6917747	TTCCTGGTGGTGGTTTTCTC
112	LOC729293	rs6570426	CATTTCTGGAACTGCCTTGG
113	SNAP25	rs6077690	CCTCCTCCATTCCTTCACAA
114	MORF4	rs4473631	TCATATGCCTGGCAGTTTACA
115	SNAP25	rs3787283	AGGGCTGCTACCAGCATAAA
116	LOC728594	rs3756450	TTGGAGACAGCAGTCAGTGG
117	SLC6A2	rs28386840	GCGGCCTTCATGGATAAATA
118	ZNF544	rs260461	GAGGCCACAAGTCCAAAATC
119	MHC II/HLA-DQA1	rs2187668	CTTAGCCACATGCCCATTTT
120	SLC6A4	rs2066713	CTTCTGAGATGGACCGCATT
121	CSMD1	rs2049306	TTGCCACTAGTTCTGAAAGCA
122	SLC1A3	rs2032893	ATCCCTATCAGGGGCAGACT
123	LOC647094	rs2028455	GCATAATGCCACAGGACCTT
124	DMRT2	rs17641078	GCCTCACACTCCTGAGATCC
125	DBH	rs1611115	ACAGGAGGGAAAAGGAAGGA
126	MMP24	rs1555322	CAACAGCTGCCATTCTGTGT
127	DSEL	rs13353224	TGGGGGTGCTAAGACAGTTT
128	C1orf125	rs12047808	GGCAAATCAAATCCAGCAGT
129	GPC5	rs10492503	GCGGAAGATTGGATAACTGC
130	C1GALT1	rs10259085	AGTCATAAGGCCGGAGTCCT
131	MET	rs10243024	AGCGATTTCTGGAAGCATGT
132	ICOS	rs4404254	CCCGGAATTGAAAGCAAAT
133	OAS1	rs3741981/rs1131454	GGATCAGGAATGGACCTCAA

Table 9: Examples of Reverse Primers Used in SNP Analysis

SNP #	Gene Symbol	rs ID	Reverse Primers (sequence 5' >3')
1	EBF1	rs1368297	CTGCCCAGTGCTTTTCATTT
2	RANTES/CCL5	rs2280788	GAGGGCAGTAGCAATGAGGA
3	RANTES/CCL5	rs2107538	GGAGTGGCAGTTAGGACAGG
4	TGFB1	rs17851976	ACCACACCAGCCCTGTTC
6	UPC2	rs659366	AGTCCCTTCTGCTGGTGAAA
7	IKBL	rs3130062	CTCTCACGCAGCTCTTCCTC
8	Apo I/Fas (CD 95)	rs1800682	AGTTGGGGAGGTCTTGAAGG
9	Apo I/Fas (CD 95)	rs3781202	AAGGGCCTTGTCTTTTAGGC
10	IL2	rs2069763	TCCTGGTGAGTTTGGGATTC
11	IL2	rs2069762	TCTTGCTCTTGTCCACCACA
12	IL10	rs1800896	CTTCCCCAGGTAGAGCAACA

SNP #	Gene Symbol	rs ID	Reverse Primers (sequence 5' >3')
13	IL4R	rs1801275	CCACATTTCTCTGGGGACAC
14	PTPRC	rs17612648	CTTTTGTGTGCCAACCTGTG
15	PTPRC	rs4915154	AACTGAAGACACTACTAGAGCAGCA
16	PD-1/PDCD1	rs11568821	AGGCAGGCACACACATGG
17	CRYAB	rs14133	GACTTGTGATCCGGGATTTG
18	CRYAB	rs762550	GGTCAACATGTCAGCACCAG
19	CRYAB	rs2234702	GGTCAACATGTCAGCACCAG
20	NDUFS5	rs2889683	AGTGGCAGACCATCCACATC
21	NDUFS5	rs6981	CTTTGACAAGGAGGTTTGTCG
22	NDUFS7	rs2074897	AGGAATCGTTCTGGGGAGAG
24	NDUFA7	rs2288414	GCTCTGTCCTTTCTCCACCA
25	NDUFA7	rs561	AGAAAGTCCCTGTGGGTGTG
26	ADAMTS14	rs4747075	CTGGCTTCTCTGGGAGGAAT
27	ADAMTS14	rs7081273	GCTTGGCTCTCAGGAGACAG
28	ADAMTS14	rs4746060	GCTTCAAAGTGCTCAAATGGT
29	NFKBIA	rs11569591	AAGGACGCACTGTGGTTAGG
29	NFKBIA	rs11569591	AAGGACGCACTGTGGTTAGG
29	NFKBIA	rs11569591	AAGGACGCACTGTGGTTAGG
30	SPP1	rs28357094	CCAAGCCCTCCCAGAATTTA
31	HLA-DR*1501	rs367398	CAGGAAACAGCTCAGACGTG
32	HLA-DR*1501	rs1800629	AAAGTTGGGGACACACAAGC
34	IL7R	rs11567685	GCCCAGGCTGGAGTACAATA
35	IL7R	rs7718919	CACACCACAGTAGGCATTCAA
36	IL7R	rs11567686	GCCCAGGCTGGAGTACAATA
37	IFNAR1	rs2257167	TTCGCCTAATTTTTCTCTCACA
38	IFNAR2	rs7279064	GACTTCCTGCCAGTGCTCTC
39	IL1B	rs1799916	AAACAGCGAGGGAGAAACTG
40	IFNGR2	rs9808753	TGTTTCCCACGGGTTTGATA
41	Apo I/Fas (CD 95)	rs2234978	CTGGGCTATGGAGCAAGACT
42	CD24	rs8734	ACCACGAAGAGACTGGCTGT
43	MEFV	rs28940577	GCTTGGGAGGCTCCTTTATT
44	CTLA4	rs231775	CCTCCTCCATCTTCATGCTC
45	CNTF	rs1800169	GCCAACAAAACATGGAAGGT
46	MHC2TA	rs3087456	CAAGCTAAGCCAACATGCAA
47	VDR	rs1544410	CAGGAATGTTGAGCCCAGTT
48	PRKCA	rs7220007	GCATAGCCTCGGAGACAGAC
49	PRKCA	rs887797	TCCCGGGTATATGATCTCCA
50	PRKCA	rs2078153	TCACCTAAGGACAGTCTAAAATTGC
51	CTLA4	rs5742909	AGCCGTGGGTTTAGCTGTTA
52	MGC33887	rs987931	GCTTGGAAGTTGCCATTCAT
53	CACNG4	rs4790896	AGCTTGCCACAGGACAGTTT
54	HELZ	rs2363846	TTGAGTTGTTGCAGCAGAGATT

SNP #	Gene Symbol	rs ID	Reverse Primers (sequence 5' >3')
55	PITPNC1	rs1318	TGCCTTTTGATGACTGGGTTA
56	PITPNC1	rs2365403	AGCAGGGAAGCACTTGAAGA
57	MC1R	rs1805009	GGTCACACAGGAACCAGACC
58	MC1R	rs1805006	TGCAGGTGATCACGTCAATG
59	PRKCA	rs1010544	CCCCAAACCCTGACTTTCAT
60	PRKCA	rs3890137	TACTGATTGAGCCCCCTTGT
61	BTNL2 (DRb1*15)	rs2076530	TTAAAGTGGCAGGAGCAGGT
62	PNMT	rs876493	CCCATTCATCCATCTCCCTTA
63	PNMT	rs3764351	CCTCACCCCCACAATCTTTA
64	TRAIL/TNFSF10	rs9880164(rs1131568 in v. 37.1)	CGAGATCAAGAGATCAAGACCA
65	PTPN22	rs2476601	TGGATAGCAACTGCTCCAAG
66	MOG	rs3130250	GCTGGAAGACACTTGGAGGA
67	MOG	rs3130253	TCCAAGAAGCCAGCTCATTT
68	SPP1	rs9138	CACACCACAAAAAGATAATCACAA
69	SPP1	rs4754	CATCAGACTGGTGAGAATCATC
70	SPP1	rs1126616	ATTCACGGCTGACTTTGGAA
71	SPP1	rs1126772	TGAACATAGACATAACCCTGAAGC
72	HLA-DRA	rs2395182	TCCACTCAAAGACACATCTTCAA
73	HLA	rs2395166	TGTGTCAGGCAATGAGGCTA
74	HLA	rs2213584	GGCATCTGAGACTATGTCTAACAGAA
75	HLA	rs2227139	GGGTTGGGGAGAAAGATATGA
76	IL1RN	rs419598	ATTGCACCTAGGGTTTGTGC
77	IL1RN	2073 Intron2 C/T (rs423904)	ATTGCACCTAGGGTTTGTGC
78	NOS2A	rs1137933	CCCTTCAATGGCTGGTACAT
79	GABBRA1	rs1805057	TGGCCTATGATGCCATCTG
80	VDR	rs731236	CTGAGAGCTCCTGTGCCTTC
81	NOS2A	rs2779248	CAGCTTCCTGGACTCCTGTC
82	IL1B	rs1143627	TTTGCTACTCCTTGCCCTTC
83	HLA-DRA	rs2239802	TGTAAGGCACATGGAGGTGA
84	IL1B	rs1143634	GTGATCGTACAGGTGCATCG
85	SPP1	rs2853744	GCTTGTTACTTAGACAAATGGCACT
87	CCR5	rs333	TGTAGGGAGCCCAGAAGAGA
88	HLA-DRA	rs3135388	TCCATACCTTGGGGTTTCAG
89	HLA	rs9268458	TGCAGGGTTTTGATACATGG
90	HLA	rs6457594	ATTTCTCCTCCACCCTCTGC
91	HLA	rs7451962	GAACGGTCCTCTCACTTCTCA
92	HLA	rs7451962	GAACGGTCCTCTCACTTCTCA
93	HLA	rs7451962	GAACGGTCCTCTCACTTCTCA
94	PNMT	rs3764351	CCTCACCCCCACAATCTTTA
95	KIF1B	rs10492972	CACATTGGAATTTGGGAAGAA
96	IGF2R	rs12202350	AGGTGAGGGGCTGAAGAAGT

SNP #	Gene Symbol	rs ID	Reverse Primers (sequence 5' >3')
97	GRIN2A	rs8049651	GTCCTTCTCCGACTGTGAGC
98	KLC1	rs8702	CATGACGGTGACCTGTTGAC
99	IL7R	rs987107	CCCCACTTCCACCAAAATTA
100	STS	rs12861247	GGATTGGCTGAACATTTTGG
101	GPC6	rs7995215	AATGGGTGGGGGTGTTATTT
102	EREG	rs1350666	GACTGAGTGCAATGCCAAAA
103	ADRA1A	rs3808585	CGCTTTTTCCACCAGGTTT
104	IL16	rs4128767	CTGGGCTCTGCTTGTTTCTC
105	ARRB2	rs7208257	AGCTGTTCCTCCCGTACCTT
106	NTF3	rs7956189	AGACTAGTGCCGAGGGTTCA
107	IL12A	rs4680534	TCGTGCAAAATCAAGGTTCA
108	SLC6A4	rs1042173	CAAGCTTGCATGGACACACT
109	FLJ34870	rs7577925	ATCTTGGCATCTCCTTGGTG
110	FCRL3	rs7528684	TGAGAAGGGCTTTGGCTTTA
111	IGF2R	rs6917747	CCCTAAGAAAGGTGCCATGA
112	LOC729293	rs6570426	AAATGGTGCTGGGAAAACTG
113	SNAP25	rs6077690	GAATAGGGGGAAAGGGGTTT
114	MORF4	rs4473631	CTTGAAGGATGCTTTCCAAGA
115	SNAP25	rs3787283	AGTTTGGTTTCCCCACACTG
116	LOC728594	rs3756450	TTTGCCCTAAATGCCAAGTC
117	SLC6A2	rs28386840	AGGGAAGGAAACCAGGAGAA
118	ZNF544	rs260461	GGAGAAAGGCAGAGGGAGAT
119	MHC II/HLA-DQA1	rs2187668	TCTCCGGTGGTAGATCTTGG
120	SLC6A4	rs2066713	TCCTGACCTCACATGATCCA
121	CSMD1	rs2049306	TTCACTTCGACCAGGATATTCA
122	SLC1A3	rs2032893	TCGGGCATTCACAATGTTTA
123	LOC647094	rs2028455	AATCAGTGCTGCTGCTTGTG
124	DMRT2	rs17641078	TCAGGACCCGATTTGTCAGT
125	DBH	rs1611115	ACAGGACCTTTGCCATCATC
126	MMP24	rs1555322	GATCCTGAGGGTGGAACTGA
127	DSEL	rs13353224	CATGAGGCTGGGAGTTAGGA
128	C1orf125	rs12047808	GGCAGGCAATACACACACAC
129	GPC5	rs10492503	CATCCCATGGATTTGTAGCC
130	C1GALT1	rs10259085	GCAAGGCATCTATCCTGGAG
131	MET	rs10243024	GATGGGTCCCCATTTTTCTT
132	ICOS	rs4404254	GCTCTACCCCATGAGAATGC
133	OAS1	rs3741981/rs1131454	GGAGAACTCGCCCTCTTTCT

Table 10 shows SNPs and associated risk alleles for MS disease severity. Presence of one or more risk alleles as indicated in Table 10 at the specified SNPs is associated with a higher probability that the subject has a greater severity of MS disease phenotype, for example: a multiple sclerosis severity score (MSSS) of 2.5 or greater; an increase in size and/or distribution of T2 brain lesions; an increased number of focal lesions in the spinal cord; an increased T2 lesion load in the brain; and/or the presence of diffuse abnormalities in the spinal cord.

Table 10:

Marker	RS	Risk allele
PGK_317	rs2107538	T
PGK_309	rs1137933	G
PGK_324	rs1318	A
PGK_066	rs2069763	G
PGK_027	rs423904	C
PGK_321	rs876493	A
PGK_169	rs10243024	G
PGK_156	rs10259085	G
PGK_310	rs1042173	A
PGK_268	rs10492503	T
KIF1B	rs10492972	G
PGK_014	rs12047808	G
PGK_154	rs12202350	A
PGK_377	rs12861247	G
PGK_332	rs13353224	A
PGK_059	rs1350666	G
PGK_358	rs1555322	A
PGK_202	rs1611115	A
PGK_186	rs17641078	G
PGK_302	rs1805009	G
PGK_328	rs2028455	G
PGK_097	rs2032893	A
PGK_176	rs2049306	A
PGK_312	rs2066713	A
NDUFS7	rs2074897	A

Marker	RS	Risk allele
BTNL2	rs2076530	G
PGK_134	rs2187668	A
MHC II	rs2213584	A
MHC II	rs2227139	C
FAS	rs2234978	T
MHC II	rs2239802	G
MHC II	rs2395182	G
PGK_350	rs260461	A
PGK_289	rs28386840	A
MHC2TA	rs3087456	G
MHC II	rs3135388	A
PGK_256	rs3741981 in NCBI db SNP build 129; Homo sapiens build 36.3 (rs1131454 in NCBI db SNP build 131; Homo sapiens build 37.1)	A
PGK_086	rs3756450	A
FAS	rs3781202	CT heterozygosity
PGK_355	rs3787283	A
PGK_181	rs3808585	A
PGK_280	rs4128767	G
PGK_036	rs4404254	G
PGK_070	rs4473631	C
PGK_051	rs4680534	A
PGK_352	rs6077690	T
HLA_M9001	rs6457594	A
PGK_150	rs6570426	T
UCP2	rs659366	C
PGK_155	rs6917747	G
PGK_304	rs7208257	A
PGK_011	rs7528684	G
PGK_030	rs7577925	A
CRYAB	rs762550	A
PGK_234	rs7956189	G

Marker	RS	Risk allele
GPC6	rs7995215	G
PGK_285	rs8049651	A
KLC1	rs8702	G
IFNGR2	rs9808753	G
IL7R	rs987107	A

EQUIVALENTS

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

All references, including patent documents, disclosed herein are incorporated by reference in their entirety, particularly for the disclosure referenced herein.

CLAIMS

1. A method of assessing a multiple sclerosis disease severity phenotype in a human subject having multiple sclerosis, the method comprising determining the genotype of the subject at one or more positions of single nucleotide polymorphism (SNP) selected from: rs2107538, rs1137933, rs1318, rs2069763, rs423904, rs876493, rs10243024, rs10259085, rs1042173, rs10492503, rs10492972, rs12047808, rs12202350, rs12861247, rs13353224, rs1350666, rs1555322, rs1611115, rs17641078, rs1805009, rs2028455, rs2032893, rs2049306, rs2066713, rs2074897, rs2076530, rs2187668, rs2213584, rs2227139, rs2234978, rs2239802, rs2395182, rs260461, rs28386840, rs3087456, rs3135388, rs3741981, rs3756450, rs3781202, rs3787283, rs3808585, rs4128767, rs4404254, rs4473631, rs4680534, rs6077690, rs6457594, rs6570426, rs659366, rs6917747, rs7208257, rs7528684, rs7577925, rs762550, rs7956189, rs7995215, rs8049651, rs8702, rs9808753 and rs987107, and/or a SNP in linkage disequilibrium with any one of said SNPs, wherein said SNPs are as disclosed in the NCBI dbSNP build 131, *Homo sapiens* genome build 37.1, and wherein the presence of:

at least one T allele at rs2107538;

at least one G allele at rs1137933;

at least one A allele at rs1318;

at least one G allele at rs2069763;

at least one C allele at rs423904;

at least one A allele at rs876493;

at least one G allele at rs10243024;

at least one G allele at rs10259085;

at least one A allele at rs1042173;

at least one T allele at rs10492503;

at least one G allele at rs10492972;

at least one G allele at rs12047808;

at least one A allele at rs12202350;

at least one G allele at rs12861247;

at least one A allele at rs13353224;

at least one G allele at rs1350666;
at least one A allele at rs1555322;
at least one A allele at rs1611115;
at least one G allele at rs17641078;
at least one G allele at rs1805009;
at least one G allele at rs2028455;
at least one A allele at rs2032893;
at least one A allele at rs2049306;
at least one A allele at rs2066713;
at least one A allele at rs2074897;
at least one G allele at rs2076530;
at least one A allele at rs2187668;
at least one A allele at rs2213584;
at least one C allele at rs2227139;
at least one T allele at rs2234978;
at least one G allele at rs2239802;
at least one G allele at rs2395182;
at least one A allele at rs260461;
at least one A allele at rs28386840;
at least one G allele at rs3087456;
at least one A allele at rs3135388;
at least one A allele at rs3741981;
at least one A allele at rs3756450;
a C allele and a T allele at rs3781202;
at least one A allele at rs3787283;
at least one A allele at rs3808585;
at least one G allele at rs4128767;
at least one G allele at rs4404254;
at least one C allele at rs4473631;
at least one A allele at rs4680534;
at least one T allele at rs6077690;

at least one A allele at rs6457594;
at least one T allele at rs6570426;
at least one C allele at rs659366;
at least one G allele at rs6917747;
at least one A allele at rs7208257;
at least one G allele at rs7528684;
at least one A allele at rs7577925;
at least one A allele at rs762550;
at least one G allele at rs7956189;
at least one G allele at rs7995215;
at least one A allele at rs8049651;
at least one G allele at rs8702;
at least one G allele at rs9808753; and/or
at least one A allele at rs987107 is indicative of the subject having a more severe multiple sclerosis disease phenotype.

2. A method according to claim 1, wherein the presence of:
the TT genotype at rs2107538;
the GG genotype at rs1137933;
the AA genotype at rs1318;
the GG genotype at rs2069763;
the CC genotype at rs423904;
the AA genotype at rs876493;
the GG genotype at rs10243024;
the GG genotype at rs10259085;
the AA genotype at rs1042173;
the TT genotype at rs10492503;
the GG genotype at rs10492972;
the GG genotype at rs12047808;
the AA genotype at rs12202350;
the GG genotype at rs12861247;

the AA genotype at rs13353224;
the GG genotype at rs1350666;
the AA genotype at rs1555322;
the AA genotype at rs1611115;
the GG genotype at rs17641078;
the GG genotype at rs1805009;
the GG genotype at rs2028455;
the AA genotype at rs2032893;
the AA genotype at rs2049306;
the AA genotype at rs2066713;
the AA genotype at rs2074897;
the GG genotype at rs2076530;
the AA genotype at rs2187668;
the AA genotype at rs2213584;
the CC genotype at rs2227139;
the TT genotype at rs2234978;
the GG genotype at rs2239802;
the GG genotype at rs2395182;
the AA genotype at rs260461;
the AA genotype at rs28386840;
the GG genotype at rs3087456;
the AA genotype at rs3135388;
the AA genotype at rs3741981;
the AA genotype at rs3756450;
the CT genotype at rs3781202;
the AA genotype at rs3787283;
the AA genotype at rs3808585;
the GG genotype at rs4128767;
the GG genotype at rs4404254;
the CC genotype at rs4473631;
the AA genotype at rs4680534;

the TT genotype at rs6077690;
the AA genotype at rs6457594;
the TT genotype at rs6570426;
the CC genotype at rs659366;
the GG genotype at rs6917747;
the AA genotype at rs7208257;
the GG genotype at rs7528684;
the AA genotype at rs7577925;
the AA genotype at rs762550;
the GG genotype at rs7956189;
the GG genotype at rs7995215;
the AA genotype at rs8049651;
the GG genotype at rs8702;
the GG genotype at rs9808753; and/or
the AA genotype at rs987107 is indicative of the subject having a more severe multiple sclerosis disease phenotype.

3. A method according to claim 1 or claim 2, wherein said more severe multiple sclerosis disease phenotype is selected from: a multiple sclerosis severity score (MSSS) of 2.5 or greater, an increase in size and/or distribution of T2 brain lesions, an increased number of focal lesions in the spinal cord, an increased T2 lesion load in the brain, and the presence of diffuse abnormalities in the spinal cord.

4. A method according to any one of the preceding claims, wherein the method comprises determining the genotype of the subject at 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of said positions of SNP.

5. A method according to any one of the preceding claims, wherein the method further comprises the measurement of at least one clinical variable.

6. A method according to claim 5, wherein the at least one clinical variable is selected from: age of the subject at onset of multiple sclerosis, gender of the subject and type of multiple sclerosis at onset of multiple sclerosis.

7. A method according to any one of the preceding claims, wherein the method comprises determining the genotype of the subject at at least rs2107538, rs1137933 and rs1318.

8. A method according to claim 7, wherein the method comprises determining the genotype of the subject at at least rs2107538, rs1137933, rs1318, rs2069763, rs423904 and rs876493.

9. A method according to any one of claims 1 to 5, wherein said more severe multiple sclerosis disease phenotype comprises a multiple sclerosis severity score (MSSS) of 2.5 or greater, and wherein the method comprises determining the genotype of the subject at at least 2 of the following positions of SNP:
rs423904, rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3756450, rs12047808, rs10259085, rs1042173, rs6077690, rs1611115, rs4473631, rs2032893, rs2066713, rs260461, rs3787283, rs6917747, rs2049306, rs12861247, rs4404254, rs4680534, rs17641078, rs2187668, rs7528684, rs7577925, rs1805009, rs3741981, rs12202350, rs28386840, rs2028455, rs10492503, rs8049651, rs13353224, rs1555322, rs10243024 and rs6570426.

10. A method according to claim 9, wherein the method comprises determining the genotype of the subject at at least the following positions of SNP: rs2107538, rs1137933, rs1318, rs2069763, rs423904 and rs876493.

11. A method according to claim 10, wherein the method further comprises determining at least 1, 2 or 3 clinical variables selected from: age of the subject at onset of multiple sclerosis, gender of the subject and type of multiple sclerosis at onset of multiple sclerosis.

12. A method according to any one of claims 1 to 5, wherein said more severe multiple sclerosis disease phenotype comprises increased size and/or distribution of T2 brain lesions,

and wherein the method comprises determining the genotype of the subject at at least 2, 3 or 4 of the following positions of SNP:
rs2213584, rs2227139, rs2076530 rs876493, rs9808753, rs2074897, rs762550, rs2234978, rs3781202, .

13. A method according to any one of claims 1 to 5, wherein said more severe multiple sclerosis disease phenotype comprises increased T2 lesion load in the brain, and wherein the method comprises determining the genotype of the subject at at least 1, 2, 3 or 4 of the following positions of SNP:
rs2107538, rs12861247, rs2074897 and rs7995215.

14. A method according to claim 13, wherein the method comprises determining the genotype of the subject at at least the following positions of SNP:
rs12861247, rs2074897 and rs7995215.

15. A method according to any one of claims 1 to 5, wherein said more severe multiple sclerosis disease phenotype comprises an increased number of focal lesions in the spinal cord, and wherein the method comprises determining the genotype of the subject at at least 1, 2, 3 or 4 of the following positions of SNP:
rs3135388, rs2395182, rs2239802, rs2227139, rs2213584, rs3087456, rs10492972, rs12202350, rs8049651, rs8702 and rs987107.

16. A method according to claim 15, wherein the method comprises determining the genotype of the subject at at least the following positions of SNP:
rs3135388, rs3087456 and rs2227139.

17. A method according to any one of claims 1 to 5, wherein said more severe multiple sclerosis disease phenotype comprises the presence of diffuse abnormalities in the spinal cord, and wherein the method comprises determining the genotype of the subject at at least 1, 2, 3 or 4 of the following positions of SNP:
rs1350666, rs3808585, rs4128767, rs6457594, rs7208257 and rs7956189.

18. A method according to any one of the preceding claims, wherein the method is carried out *in vitro* using a nucleic acid-containing sample that has been obtained from the subject.

19. A method according to any one of the preceding claims, wherein the genotype of the subject at said one or more positions of SNP is determined indirectly by determining the genotype of the subject at a position of SNP that is in linkage disequilibrium with said one or more positions of SNP.

20. A method according to any one of the preceding claims, wherein determining the genotype of the subject at said one or more positions of SNP comprises:

(i) extracting and/or amplifying DNA from a sample that has been obtained from the subject;

(ii) contacting the DNA with an array comprising a plurality of probes suitable for determining the identity of at least one allele at a position of SNP as defined in claim 1.

21. A method according to claim 20, wherein the array is a DNA array, a DNA microarray or a bead array.

22. A method according to claim 20 or claim 21, wherein said plurality of probes are selected from the probes listed in Table 7.

23. A method according to any one of the preceding claims, wherein the method comprises amplifying DNA from a sample that has been obtained from the subject, and wherein said amplifying comprises contacting the DNA with at least one forward primer as listed in Table 8.

24. A method according to any one of the preceding claims, wherein the method comprises amplifying DNA from a sample that has been obtained from the subject, and wherein said amplifying comprises contacting the DNA with at least one reverse primer as listed in Table 9.

25. An array of probes for use in a method according to any one of claims 1 to 24, wherein the array comprises:

at least 5, 10, 15, 20, 50 or more nucleic acid probes suitable for determining the identity of at least one allele at a position of SNP as defined in claim 1; and

a solid support on which said probes are immobilised,

wherein said probes comprise at least 50% of the total number of nucleic acid probes in the array.

26. An array according to claim 25, wherein said probes are selected from the probes listed in Table 7.

27. A method of evaluating disease severity in a patient having multiple sclerosis, the method comprising

obtaining a DNA sample from the patient, and

determining the presence or absence of two or more single nucleotide polymorphisms (SNPs) associated with severity of the disease,

wherein the presence of two or more SNPs associated with severity of the disease indicates a likelihood of increased disease severity.

28. The method of claim 27, wherein the two or more SNPs associated with the disease severity are selected from the group consisting of the SNPs listed in Table 3, Table 5, Table 5A and Table 3A, and rs2076530.

29. The method of claim 27, wherein the two or more SNPs associated with the disease severity comprise SNPs in PNMT, IL1R, CCL5, IL2, PITPNC1 or NOS2A.

30. The method of claim 27, wherein the two or more SNPs associated with the disease severity are selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763 and rs2107538.

31. The method of claim 27, wherein the two or more SNPs associated with the disease severity are selected from the group consisting of the SNPs listed in Table 5, Table 5A and rs2076530.

32. The method of claim any one of claims 27 to 31, wherein the presence or absence of two or more SNPs associated with severity of the disease is determined using a method comprising a gene chip, gene array, bead array, RFLP analysis, and/or sequencing.

33. The method of claim 27, wherein the two or more SNPs associated with the disease severity are selected from the group consisting of, rs876493, rs1137933, rs1318, rs2069763 and rs2107538.

34. The method of claim 27, wherein the two or more SNPs associated with the disease are selected from the group consisting of rs1137933, rs1318, rs2069764 and rs2107538.

35. The method of claim 27, wherein the SNP is associated with increased T2 lesion load in the brain.

36. The method of claim 27, wherein the SNP is associated with an increased number of focal spinal cord abnormalities.

37. A method as defined in claim 27, wherein the two or more SNPs are in linkage disequilibrium an SNP as defined in claim 28.

38. The method of claim 27, wherein the SNP is associated with spatial distribution of T2 brain lesions.

39. The method of claim 27, wherein the SNP is associated with the presence of diffuse abnormalities.

40. The method of claim 27, further comprising the measurement of one or more clinical variables.

41. The method of claim 40, wherein the clinical variable comprises age of onset, gender, and/or type of onset of disease.

42. The method of claim 27, wherein disease severity is based on an MS severity scale.

43. The method of claim 42, wherein the MS severity scale is based on the Multiple Sclerosis Severity Score (MSSS) test, the Kurtzke Expanded Disability Status Scale (EDSS), or the Multiple Sclerosis Functional Composite (MSFC) measure.

44. The method of any one of claims 27 to 43,A1-A21 wherein the presence or absence of at least 6 SNPs is determined.

45. The method of claim 27, wherein the two or more SNPs are selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456.

46. The method of claim 27, wherein at least one of the SNPs is in linkage disequilibrium with a SNP selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456.

47. The method of claim 27, wherein the method further comprises use of one or more probe sets listed in Table 7.

48. The method of claim 27, wherein the method further comprises use of at least one forward primer from Table 8 and one reverse primer from Table 9.

49. A method of designing a treatment regimen for a patient having multiple sclerosis, the method comprising

obtaining a DNA sample from the patient,

determining the presence or absence of two or more single nucleotide polymorphisms (SNPs) associated with severity of the disease, wherein the presence of two

or more SNPs associated with severity of the disease indicates a likelihood of increased disease severity, and

designing the treatment regimen based on the presence or absence of the SNPs associated with the disease.

50. The method of claim 49, wherein said treatment regimen comprises early or elevated doses of glatiramer acetate, vitamin D, interferon beta-1a or -1b, natalizumab, mitoxantrone, and/or corticosteroids.

51. A method of treating a patient having a prognosis of increased disease severity, comprising early or elevated doses of glatiramer acetate, vitamin D, interferon beta-1a or -1b, natalizumab, mitoxantrone, and/or corticosteroids.

52. A method of identifying SNPs associated with severity of symptoms in multiple sclerosis, comprising

obtaining a DNA sample from a patient having multiple sclerosis,

identifying SNPs in the DNA,

wherein the SNPs comprise two or more of the SNPs listed in Table 1,

performing an MRI on the patient to determine spatial distribution of T2 brain lesions, T2 lesion load and/or number of spinal cord lesions,

comparing identified SNPs with the spatial distribution of T2 brain lesions, T2 lesion load and/or number of spinal cord lesions, and

identifying the SNPs that correlate with spatial distribution of T2 brain lesion, T2 lesion load and/or number of spinal cord lesions, wherein the SNPs that correlate with spatial distribution of T2 brain lesions, T2 lesion load and/or number of spinal cord lesions, are SNPs associated with severity of symptoms in multiple sclerosis.

53. The method of claim 52, wherein at least one of the SNPs is in linkage disequilibrium with a SNP listed in Table 1.

54. The method of claim 52, wherein identifying SNPs associated with severity of symptoms in multiple sclerosis further comprises consideration of clinical data.

55. A method of evaluating disease severity, as measured using the Multiple Sclerosis Severity Score (MSSS) test, the Kurtzke Expanded Disability Status Scale (EDSS), and/or the Multiple Sclerosis Functional Composite measure (MSFC),
in a patient having multiple sclerosis, the method comprising
obtaining a DNA sample from the patient, and
determining the presence or absence of two or more single nucleotide polymorphisms (SNPs),
wherein said SNPs comprise two or more of the SNPs listed in Table 1, and
wherein the presence of said two or more SNPs indicates a likelihood of increased disease severity.

56. The method of claim 55, wherein evaluating disease severity further comprises consideration of clinical data.

57. The method of claim 55 wherein at least one of the SNPs is in linkage disequilibrium with a SNP listed in Table 1.

58. A method of evaluating the severity of spinal cord lesions in a patient having multiple sclerosis, the method comprising
obtaining a DNA sample from the patient, and
determining the presence or absence of two or more single nucleotide polymorphisms (SNPs) associated with spinal cord lesions,
wherein the presence of two or more SNPs associated with spinal cord lesions indicates a likelihood of increased disease severity.

59. The method of claim 58, wherein the two or more SNPs are selected from the group consisting of rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456.

60. The method of claim 58, wherein one of the SNPs is rs3135388.

61. The method of claim 58 wherein the two or more SNPs are selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456.

62. The method of claim 58, wherein at least one of the SNPs is in linkage disequilibrium with a SNP selected from the group consisting of 2073 Intron2 C/T (rs423904), rs876493, rs1137933, rs1318, rs2069763, rs2107538, rs3135388, rs2395182, rs2239802, rs2227139, rs2213584 and rs3087456.

63. A method of prognosing the likelihood of T2 lesions and/or T2 lesion load in a patient having multiple sclerosis, the method comprising

obtaining a DNA sample from the patient, and

determining the presence or absence of SNP rs2107538,

wherein the presence of SNP rs2107538 indicates a likelihood of T2 lesions and/or T2 lesion load in the patient.

64. A method according to any one of claims 27 to 63, wherein determining the presence or absence of SNPs comprises

(a) providing, for each genetic variation to be genotyped, at least 2 oligonucleotide probe pairs, wherein:

(i) one pair consists of probes 1 and 2, and the other pair consists of probes 3 and 4;

(ii) one probe in each pair is capable of hybridising to genetic variation A and the other probe in each pair is capable of hybridising to genetic variation B;

(iii) each probe is provided in replicates; and

(iv) the probe replicates are each coupled to a solid support;

(c) amplifying and detectably labelling the target DNA;

(d) contacting the target DNA with the probes under conditions which allow hybridisation to occur, thereby forming detectably labeled nucleic acid-probe hybridisation complexes,

(e) determining the intensity of detectable label at each probe replica position, thereby obtaining a raw intensity value;

(f) optionally amending the raw intensity value to take account of background noise, thereby obtaining a clean intensity value for each replica; and

(g) applying a suitable algorithm to the intensity data from

(e) or (f), thereby determining the genotype with respect to each genetic variation, wherein application of the algorithm comprises calculating an average intensity value from the intensity values for each of the replicas of each probe and wherein the algorithm uses three Fisher linear functions that characterize each of the three possible genotypes AA, AB or BB for the genetic variation.

65. A kit for evaluating severity of disease in a subject having multiple sclerosis, the kit comprising:

(i) at least one set of probes listed in Table 7; optionally

(ii) instruction for genotyping analysis as described in claim 64; and optionally

(iii) instructions for determining the severity MS phenotype from the outcomes.

66. A PCR amplification kit comprising at least one pair of PCR primers from tables 8 and 9, a thermostable polymerase, dNTPs, a suitable buffer, and optionally instructions for use.

Figure 1

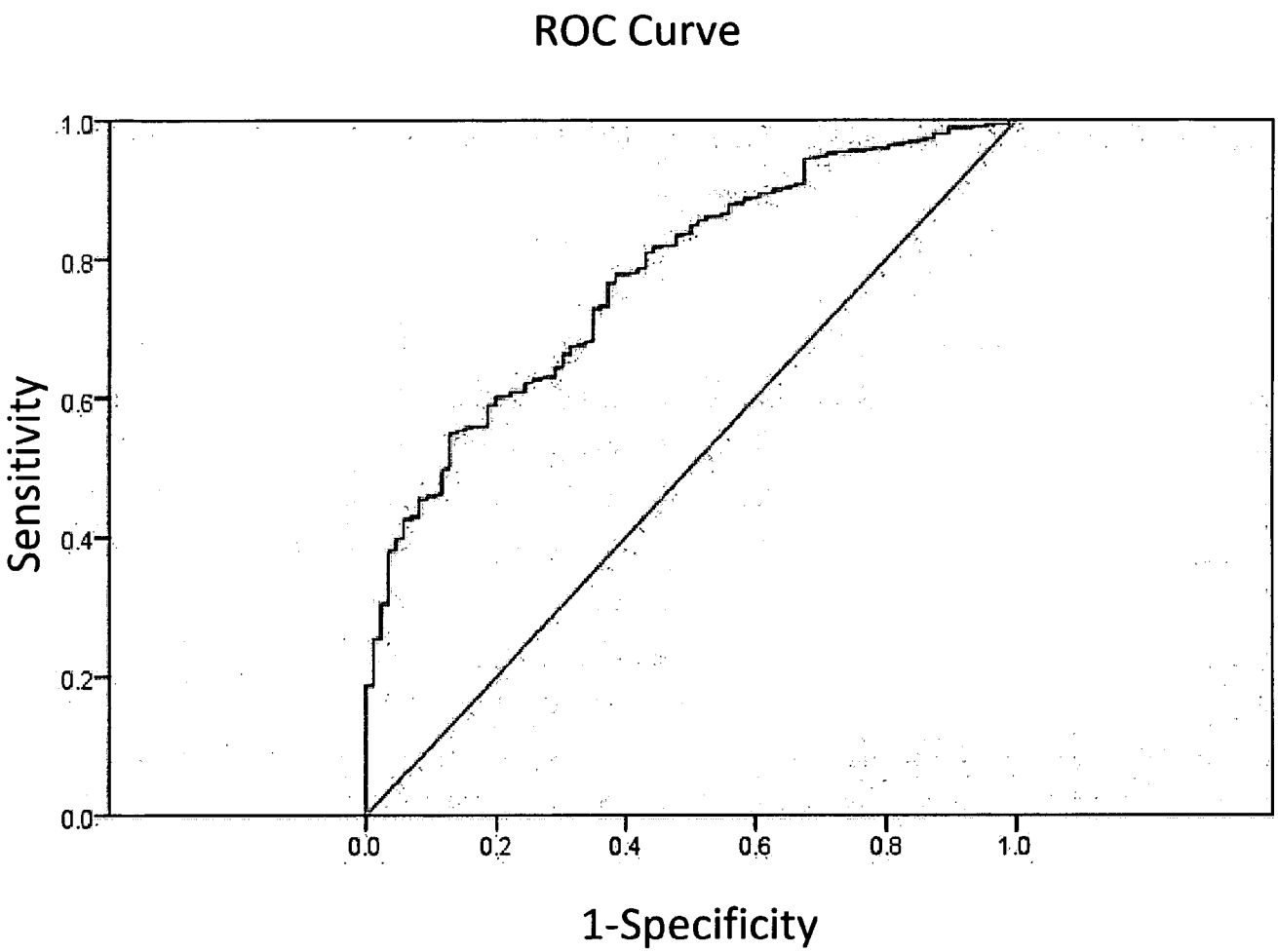
ROC Curve
Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity

Figure 2

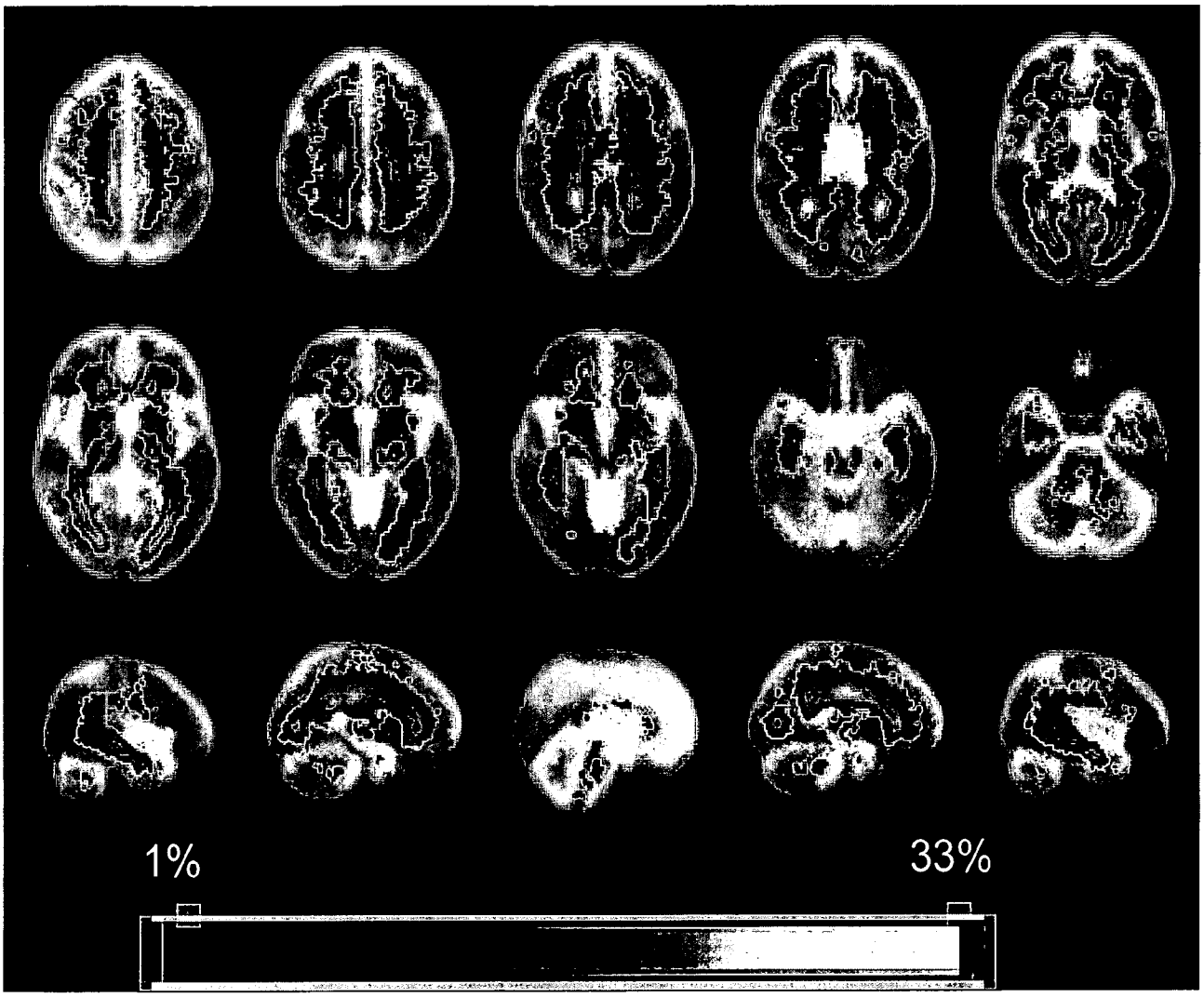

Figure 3

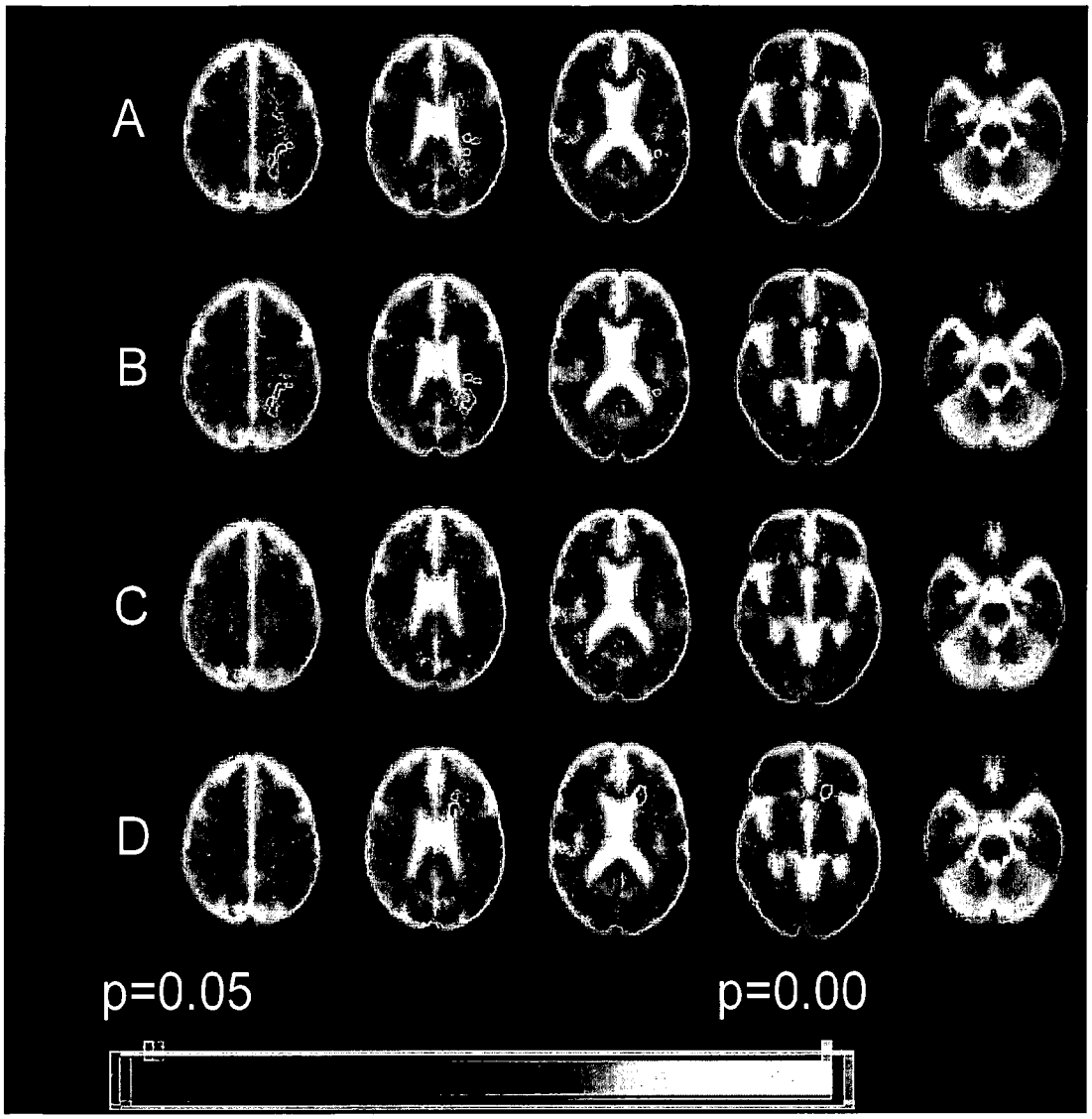

Figure 4

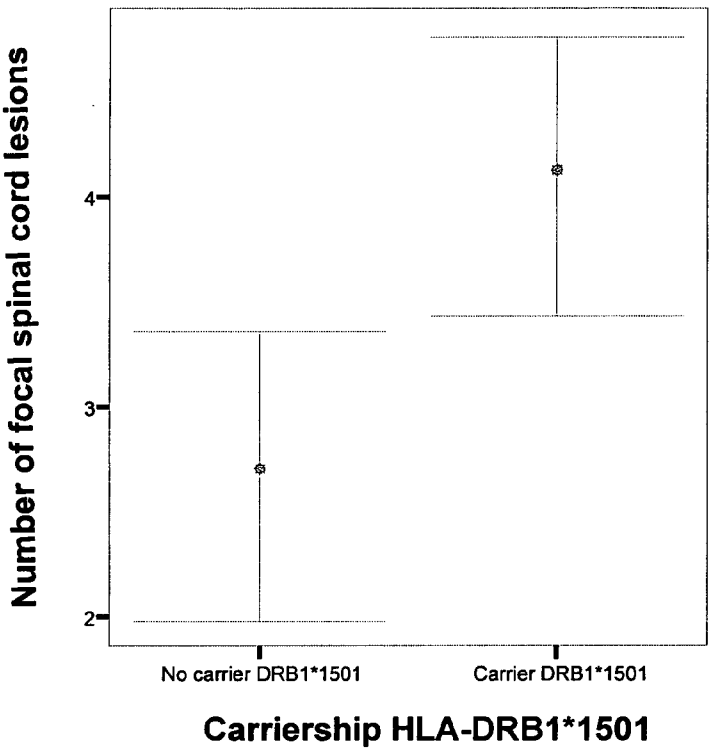
Number of focal spinal cord lesions
4
3
2
No carrier DRB1*1501
Carrier DRB1*1501
Carriership HLA-DRB1*1501

Figure 5

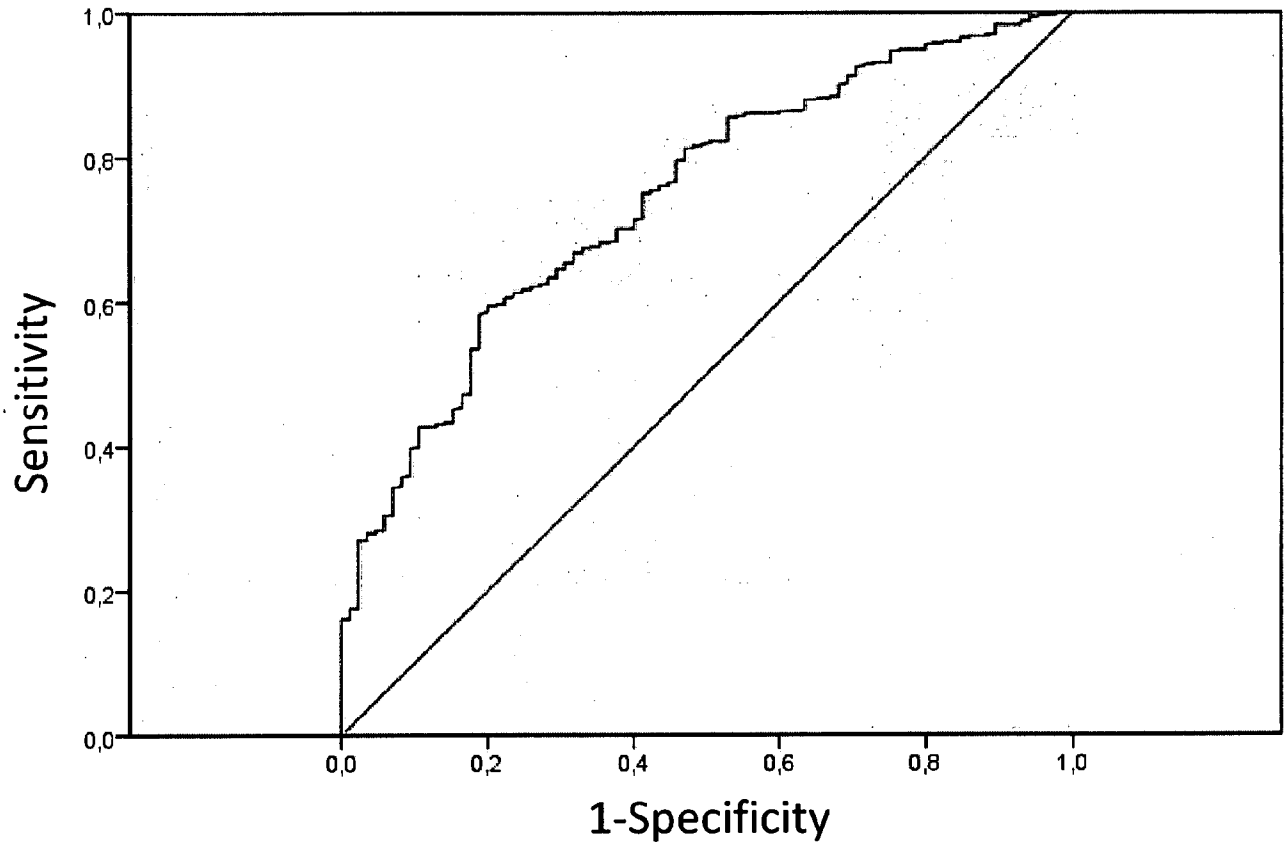

Figure 6

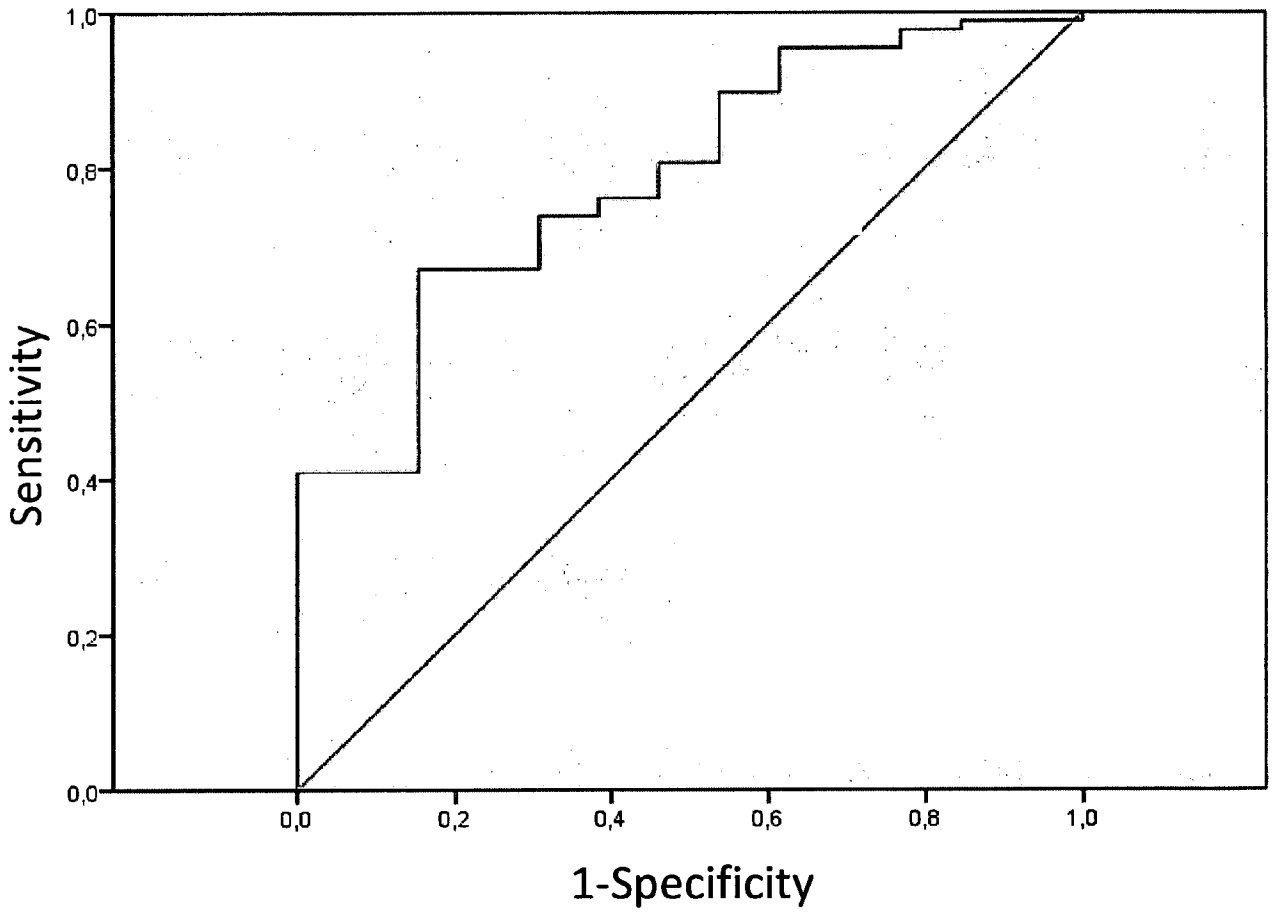

Figure 7