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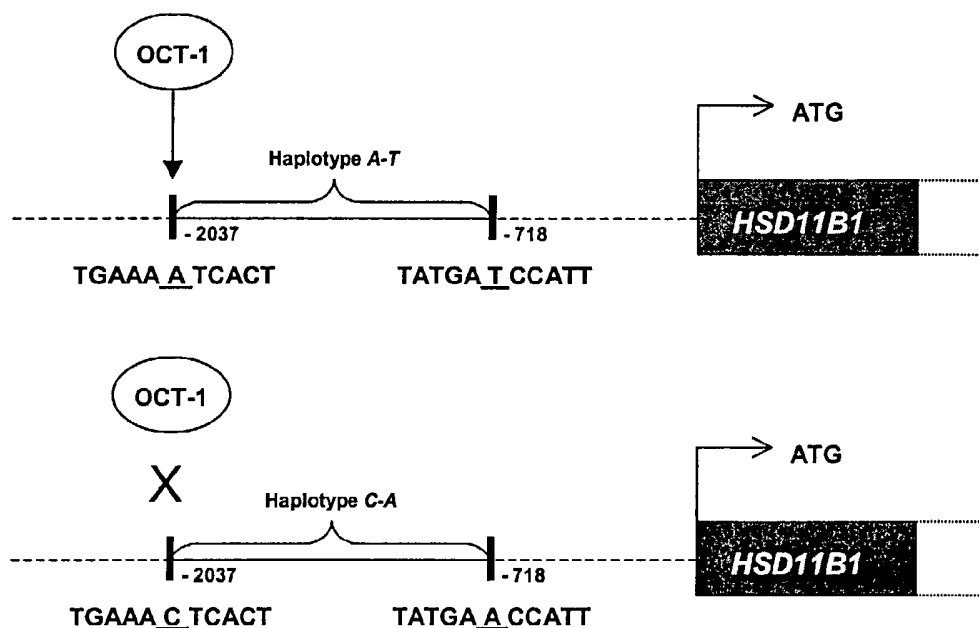
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(54) Title: DIAGNOSTIC POLYMORPHISM OF 11B-HYDROXYSTEROID DEHYDROGENASE USEFUL FOR IDENTIFYING RISK OF DEVELOPING ALZHEIMER'S DISEASE



(57) Abstract: Based on the unexpected association of a polymorphism in the gene coding for HSD11B1 with increased genetic risk for a neurodegenerative disease, in particular Alzheimer's disease, the present invention provides a method of diagnosing or prognosticating such a disease, or determining the propensity or predisposition of a subject to develop such a disease. The method comprises detecting the presence or absence of a single nucleotide polymorphism in the gene coding for HSD11B1.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Diagnostic Polymorphism of 11 β -Hydroxysteroid Dehydrogenase Useful for Identifying Risk of Developing Alzheimer's Disease

The present invention relates to methods of diagnosing, prognosticating and monitoring neurodegenerative diseases in a subject, based on the identification of the genetic association of HSD11B1 gene polymorphisms with increased genetic risk for a neurodegenerative disease, in particular Alzheimer's disease.

Neurodegenerative diseases, in particular Alzheimer's disease, have a severely debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. Alzheimer's disease is the most common age-related neurodegenerative condition affecting about 10 % of the population over 65 years of age and up to 45 % over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60:139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of elderly persons ("aging of the baby boomers") in developed countries. Alzheimer's disease (AD) is the major neurodegenerative disorder of the elderly, and is characterized by progressive cognitive deficits such as impairment of memory and orientation. The etiology of sporadic AD, which accounts for the majority of all AD cases, is multifactorial, and to date, the $\epsilon 4$ allele of the gene encoding apolipoprotein E (APOE) is the only well established genetic risk factor (Saunders et al., *Neurology* 1993, 43:1467-72). AD pathology is characterized by large extracellular β -amyloid (A β) plaques and tau-containing intraneuronal neurofibrillary tangles, which induce neuronal death and synaptic loss. Hippocampal neurons are among the first cells to degenerate in the brain of patients affected by AD (Ball et al., *Lancet* 1985, 1:14-6).

The hippocampus, which is an important brain region for memory (Squire, *Psychol Rev* 1992, 99:195-231), contains a high density of glucocorticoid receptors (McEwen et al., *Psychol Rev* 1986, 66:1121-88; Seckl et al., *Brain Res* 1991, 561:332-7). Glucocorticoids, i.e. steroid hormones released by the adrenal cortex, are known to influence cognitive functions. Acute elevation of glucocorticoid levels enhances memory consolidation (Buchanan and Lovallo, *Psychoneuroendocrinology* 2001, 26:307-17; Roozendaal, *Psychoneuro-endocrinology* 2000, 25:213-38) but impairs memory retrieval (de Quervain et al., *Nature* 1998, 394:787-90; de Quervain et al.,

Nat Neurosci 2000, 3:313-4; Wolf et al., *Behav Neurosci* 2001, 115:1002-11). Studies investigating the effects of chronically elevated glucocorticoid levels typically found impaired memory function in Cushing's syndrome, depression and senescence, which were associated with hippocampal atrophy (Starkman et al., *Biol Psychiatry* 1992, 32:756-65; Sheline et al., *Proc Natl Acad Sci USA* 1996, 93:3908-13; Lupien et al., *Nat Neurosci* 1998, 1:69-73). Chronic glucocorticoid excess leads to disruption of synaptic plasticity, atrophy of dendritic processes, reduced neuronal ability to survive a variety of coincident insults, and overt neuron death (for review: Sapolsky, *Exp Gerontol* 1999, 34:721-32). Increased circulating cortisol concentrations are consistently found in AD (Davis et al., *Am J Psychiatry* 1986, 143:300-5; Hartmann et al., *Neurobiol Aging* 1997, 18:285-9; Hatzinger et al., *Neurobiol Aging* 1995, 16:205-9; O'Brien et al., *Psychol Med* 1996, 26:7.14; Pascualy et al., *Biol Psychiatry* 2000, 48:247-54). Recently, high cortisol levels in cerebrospinal fluid were associated with increased frequency of the APOE4 allele in AD patients (Peskind et al., *Neurology* 2001, 56:1094-8). Moreover, glucocorticoids enhance the AD-associated oxidative neuronal damage (Behl, *Exp Gerontol* 1998, 33:689-96). On the other hand, low glucocorticoid levels may lead to increased AD-associated inflammatory processes which are involved in tissue destruction (Aisen et al., *Am J Psychiatry* 1994, 151:1105-1113). Taken together, it is conceivable that the glucocorticoid system is implicated in the pathophysiology of AD. This possibility led us to hypothesize that polymorphisms in genes involved in the regulation of the glucocorticoid system may influence the risk for AD.

To date, case-control association studies mostly follow a candidate-gene approach by examining one gene at a time. Although powerful, this approach ignores the genetic complexity of sporadic AD, which is caused by variations in several genes, each with a small effect on overall disease risk. Moreover, this marker-by-marker procedure causes severe inflation of the type I statistical error (i.e. false positive results) due to multiple testing in one population and overlooks possible interactions between susceptibility genes. Recently, Hoh et al. (*Genome Res* 2001, 11:2115-9) introduced a novel method (set-association approach) for the accurate evaluation of several sets of polymorphic markers throughout the genome, resulting in a powerful single genomewide test statistic. To elucidate the influence of the human glucocorticoid system on the genetic risk for AD we used the set-association method and investigated, in a large multiethnic case-control study, eleven single nucleotide polymorphisms (SNPs) in genes involved in the regulation of the glucocorticoid system: Corticotropin releasing hormone (CRH), corticotropin releasing hormone binding protein (CRHBP), ACTH-receptor (MC2R), 11 β -hydroxysteroid

dehydrogenase type 1 and 2 (HSD11B1, HSD11B2), glucocorticoid receptor (NR3C1), glucocorticoid modulatory element binding protein 1 and 2 (GMEB1, GMEB2), glucocorticoid receptor DNA binding factor 1 (GRLF1), nuclear receptor coactivator 2 (NCOA2). In addition we investigated one SNP each in the genes coding for calcium/calmodulin-dependent protein kinase 1G (CAMK1G) and interferon regulatory factor 6 (IRF6).

It is crucial to expand the pool of potential drug targets and diagnostic markers. Therefore, it is an object of the present invention to provide methods of diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease. A further objective of the present invention is to provide methods of monitoring the progression of such a disease and of evaluating a treatment for it. This objective is based on the identification of the genetic association of the gene coding for 11 β -hydroxysteroid dehydrogenase type 1 (HSD11B1) with an increased risk for Alzheimer's disease. The objective of the present invention has been solved by the methods and kits according to the features of the independent claims. Further preferred embodiments of the present invention are defined in the subclaims thereto.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. "Dysregulation" shall mean an upregulation or downregulation of gene expression. A gene product comprises either RNA or protein

and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "ORF" is an acronym for "open reading frame" and refers to a nucleic acid sequence that does not possess a stop codon in at least one reading frame and therefore can potentially be translated into a sequence of amino acids. "Regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids, natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or inorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or "primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular

sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. As used herein, "homolog or homology" is a term used in the art to describe the relatedness of a nucleotide or peptide sequence to another nucleotide or peptide sequence, which is determined by the degree of identity and/or similarity between said sequences compared. The term "variant" as used herein refers to any polypeptide or protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention. Furthermore, the term "variant" shall include any shorter or longer version of a polypeptide or protein. "Variants" shall also comprise a sequence that has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of HSD11B1. "Variants" of HSD11B1 include, for example, proteins and molecules with conservative amino acid substitutions in highly conservative regions. "Proteins and polypeptides" of the present invention include variants, fragments and chemical derivatives of HSD11B1. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants. The term "isolated" as used herein is considered to refer to molecules that are removed from their natural environment, i.e. isolated from a cell or from a living organism in which they normally occur, and that are separated or essentially purified from the coexisting components with which they are found to be associated in nature. This notion further means that the sequences encoding such molecules can be linked by the hand of man to polynucleotides, to which they are not linked in their natural state, and that such molecules can be produced by recombinant and/or synthetic means. Even if for said purposes those sequences may be introduced into living or non-living organisms by methods known to those skilled in the art, and even if those

sequences are still present in said organisms, they are still considered to be isolated.

The term "polymorphism" refers to the existence of more than one form of a gene or portion of a gene. It refers to a genetic variation in a nucleotide sequence at a given nucleotide position in the genome, within a given population, and a frequency usually exceeding 1%. Regions harboring polymorphisms may be a given gene region, coding or non-coding portions of the gene, or even intergenic regions, and are designated as "polymorphic regions". They may cause differences in the nucleotide sequences as well as in the polypeptide sequences, in protein modifications, gene and protein expression processes and DNA replication. The term "single nucleotide polymorphism (SNP)" refers to a polymorphic variation in a nucleotide sequence at a given single nucleotide position in the genome. Single nucleotide polymorphisms may include any single base changes such as a deletion, insertion, or a base exchange. A single nucleotide polymorphism may cause a change in the encoded polypeptide sequence as well. A particular SNP may be indicative for a disease state, a specific feature, or for the risk of developing a disease. A "locus" of a gene refers to a unique position on a chromosome at which the genetic information lies and includes coding sequences, intervening sequences, and regulatory elements of the given gene. The distance between the loci of genes, the map distance, is expressed either in physical terms, or in genetic terms (recombination frequency). It is said that a gene maps to a specific locus on a chromosome. The locus at which the polymorphic variation occurs is the "polymorphic site or polymorphic marker". The term "allele" or "allelic variant" refers to one of several alternative forms of a gene, or a portion thereof, typically having particular features which result in a particular phenotype. The term "allele" includes any inherited variation in the DNA sequence of a gene located at a given position in the genome. "Allele frequency" or "gene frequency" refers to the frequency with which a given allele is present at a given locus in a given population. It refers explicitly to the frequency of an allele in a population and not to the individual genotypes. The frequency distribution of two alleles in a population is following the formula $(p+q)^2 = 1.0$, and the corresponding genotypes in the population are calculated according to $p^2+2pq+q^2 = 1.0$.

An individual or a subject is "homozygous" when two alleles of a given gene of a diploid organism are identical in respect to a given variation or polymorphism. By "heterozygous" is meant that the two alleles at a given locus are different. In the present invention, the terms "risk", "susceptibility", "propensity", and "predisposition" are tantamount and are used with respect to the probability of developing a

neurodegenerative disease, preferably Alzheimer's disease. A "haplotype" refers to the polymorphisms located on a single DNA strand, it refers to a series of alleles at several closely linked gene loci on a single chromosome. Thus "haplotyping" refers to the identification of polymorphisms on a single DNA strand. "Genotype" is the genetic constitution of an individual or a cell, the types of alleles found at a given locus. "Linkage" in terms of genetics, refers to gene loci on the same chromosome, localized in a certain distance from each other, so that an independent segregation is not necessarily the case. The closer the gene loci lie to each other, the less frequently they are separated by recombination (crossing-over event between homologous chromosomes) and the higher the probability for linkage. Loci that are physically very close to each other, i.e. recombination between them will usually not occur, tend to be inherited together. The object of "linkage analysis" is to determine whether two loci tend to cosegregate more often than they would, if they were not physically close together. It is to estimate the recombination fraction, to test if the value is less than 0.5 (loci located on different chromosomes), and whether an observed deviation from 0.5 is statistically significant. The ratio of the probability that two gene loci are genetically linked, to the probability that they are not genetically linked, is referred to as "odds ratio". If the odds for linkage exceed or reach a minimal value (ratio of 1000:1), it is assumed that two gene loci are linked. The "LOD score" (logarithm of the odds) refers to the logarithm of the calculated odds ratio, and is the likelihood of a given disease, or phenotype to be localized within the genetic region examined. The formula for determining the lod score is $Z(\theta) = \log_{10} [L(\theta)] - \log_{10} [L(\theta=0.5)]$. "Linkage disequilibrium (LD)" refers to alleles which are nonrandomly associated at closely linked gene loci and operates over distances less than 1 cM. This association of alleles is not necessarily a consequence of close linkage. Allelic association will be shown only if the alleles mark conserved ancestral chromosomal segments, which is usually due to founder effects. The term "positional candidate gene" refers to a gene which is considered as a possible locus for a given disease. The assumption is based on known properties as function, expression pattern, chromosomal location etc.. The term "susceptibility gene" refers to a gene locus which is associated with a given disease and which is a major risk factor for developing said given disease. A "genetic association study" is meant to be a test for differences in the distribution of alleles between unrelated affected subjects (patients diagnosed with a given disease) and controls (healthy subjects). It is tested whether a genetic variant (SNP of a gene) increases disease risk, i.e. is associated with a trait. LD may be also tested,

whereby an increased prevalence of a characteristic set of SNP alleles in affected subjects will be identified.

The term 'AD' shall mean Alzheimer's disease. "AD-type neuropathology" as used herein refers to neuropathological, neurophysiological, histopathological and clinical hallmarks as described in the instant invention and as commonly known from state-of-the-art literature (see: Iqbal, Swaab, Winblad and Wisniewski, *Alzheimer's Disease and Related Disorders (Etiology, Pathogenesis and Therapeutics)*, Wiley & Sons, New York, Weinheim, Toronto, 1999; Scinto and Daffner, *Early Diagnosis of Alzheimer's Disease*, Humana Press, Totowa, New Jersey, 2000; Mayeux and Christen, *Epidemiology of Alzheimer's Disease: From Gene to Prevention*, Springer Press, Berlin, Heidelberg, New York, 1999; Younkin, Tanzi and Christen, *Presenilins and Alzheimer's Disease*, Springer Press, Berlin, Heidelberg, New York, 1998). Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, argyrophilic grain dementia and other tauopathies, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, age-related macular degeneration, narcolepsy, motor neuron diseases, prion diseases, traumatic nerve injury and repair, and multiple sclerosis.

In one aspect, the invention features a method for diagnosing or prognosticating a neurodegenerative disease in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease. The method comprises detecting in a sample obtained from said subject the presence or absence of a variation in the gene coding for 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD-1; HSD11B1), wherein the presence of a variation in said gene in said subject indicates a diagnosis or prognosis of a neurodegenerative disease, or an increased propensity or predisposition of developing a neurodegenerative disease as compared to a subject who does not carry a variation in said gene. A variation in the gene coding for HSD11B1 can be understood as any alteration in the naturally occurring nucleic acid sequence of the said gene, i.e. any alteration from the wildtype.

In a preferred embodiment of the herein claimed methods of diagnosing, prognosticating and monitoring, of kits, recombinant animals, molecules, assays,

and uses of the instant invention, said 11 β -hydroxysteroid dehydrogenase gene is the gene coding for the 11 β -hydroxysteroid dehydrogenase type 1, also termed corticosteroid 11-beta-dehydrogenase isozyme 1 (EC1.1.1.146), or 11-beta-HSD-1, or HSD11B1, or HSD11, or HSD11L, or 11-DH, mapping to human chromosome 1 chromosomal band q32.2, located in genomic sequence AL031316 and AL022398 (Genbank protein identification number: AAH12593; SwissProt: P28845; Genbank mRNA accession number: BC012593; Tannin et al., *J. Biological Chemistry* 1991, 266:16653-16658). In the instant invention, said 11 β -hydroxysteroid dehydrogenase type 1 gene or 11 β -hydroxysteroid dehydrogenase type 1 protein is also generally referred to as the 11 β -HSD-1 or HSD11B1 gene, or 11 β -HSD-1 or HSD11B1 protein, or just 11 β -HSD-1 or HSD11B1.

In a preferred embodiment, the variation in the gene coding for HSD11B1 is a single nucleotide polymorphism located on human chromosome 1, chromosomal position 198633436, located in the promoter region at position -2037 bases relative to the start codon (single nucleotide polymorphism identification number: rs846911). In a further preferred embodiment, the variation is a C to A transversion, hereinafter also referred to as the A-allele. However, it is a preferred embodiment of the instant invention that another polymorphism (SNP rs860185 at position -718 bases relative to the start codon) in complete linkage equilibrium with SNP rs846911 may also act independently as a diagnostic polymorphism according to the claims and methods of the present invention.

In one further preferred embodiment of the herein claimed methods of diagnosing, prognosticating and monitoring, kits, recombinant animals, molecules, assays, and uses of the instant invention, said neurodegenerative disease or disorder is Alzheimer's disease, and said subjects suffer from Alzheimer's disease.

The method according to the present invention may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease. Consequently, the method, according to the present invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted.

Determining the presence or absence of a polymorphism or variation in the gene coding for HSD11B1 may comprise determining a partial nucleotide sequence of the

DNA from said subject, said partial nucleotide sequence indicating the presence or absence of said polymorphism or variation. It may further be preferred to perform a polymerase chain reaction with the DNA from said subject to determine the presence or absence of said polymorphism or variation. Such techniques are known to those skilled in the art (see Lewin B, *Genes V*, Oxford University Press, 1994).

The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences of the HSD11B1 gene, or fragments, or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and/or variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects. Whether an amplification results in a specific nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimer's disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a preferred embodiment of the invention, the sample taken for genetic analysis comprises DNA obtained from body fluids, tissues, or any suitable cells of the body readily available. Preferably, the sample is a blood sample. However, the sample may also consist of body fluids such as saliva, urine, serum plasma, mucus, or cerebrospinal fluid.

In a further aspect, the invention features a method for diagnosing or prognosticating a neurodegenerative disease in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, comprising determining a level, or an activity, or both said level and

said activity, of at least one substance which is selected from the group consisting of a transcription product of the gene coding for HSD11B1, and/or a translation product of the gene coding for HSD11B1, and/or a fragment, or derivative, or variant thereof in a sample from said subject and comparing said level, and/or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby diagnosing or prognosticating a neurodegenerative disease in said subject, or determining the propensity or predisposition of said subject to develop a neurodegenerative disease.

In another aspect, the present invention provides a method of monitoring the progression of a neurodegenerative disease in a subject, comprising determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of the gene coding for HSD11B1, or a translation product of the gene coding for HSD11B1, and/or a fragment, or derivative, or variant thereof in a sample from said subject; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby monitoring the progression of a neurodegenerative disease in said subject.

In a further aspect, the present invention provides a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of the gene coding for HSD11B1, or a translation product of the gene coding for HSD11B1, and/or a fragment, or derivative, or variant thereof in a sample obtained from a subject being treated for a neurodegenerative disease; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby evaluating said treatment for a neurodegenerative disease.

In a preferred embodiment of the invention, the sample to be analyzed for determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of the gene coding for HSD11B1, or a translation product of the gene coding for HSD11B1, and/or a fragment, or derivative, or variant thereof is taken from the group comprising body fluid, preferably cerebrospinal fluid, saliva, urine, mucus,

blood, or serum plasma, or a tissue, or cells like skin fibroblasts. Most preferably, the sample is taken from cerebrospinal fluid.

Preferably, the methods of diagnosis, prognosis, monitoring the progression or evaluating a treatment for a neurodegenerative disease, according to the instant invention, can be practiced *ex corpore*, and such methods preferably relate to samples, for instance, body fluids or cells, removed, collected, or isolated from a subject or patient.

In a preferred embodiment of the invention, the reference value of a level, or an activity, or both said level and said activity, of a transcription product of the gene coding for HSD11B1, or a translation product of the gene coding for HSD11B1, and/or a fragment, or derivative, or variant thereof is that in a sample from a subject not suffering from a neurodegenerative disease.

The determination of a level of transcription products of the gene coding for HSD11B1 can be performed in a sample from a subject using Northern blots with probes specific for said gene. Another preferred method of measuring said level is by quantitative PCR with primer combinations which amplify said gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. It might further be preferred to measure transcription products by means of chip-based microarray technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000). An example of an immunoassay is the detection and measurement of enzyme activity as disclosed and described in the patent application WO 02/14543.

Furthermore, a level and/or an activity of a translation product of the gene coding for HSD11B1 and/or of a fragment, or derivative, or variant thereof, and/or a level of activity of said translation product of the HSD11B1 gene, and/or of a fragment, or derivative, or variant thereof, can be detected using a Western blot analysis, an immunoassay, an enzyme activity assay, and/or a binding assay. These assays can measure the amount of binding between said translation product and an anti-polypeptide antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-polypeptide antibody or a secondary antibody which binds the anti-polypeptide antibody. In

addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R, *Immunodiagnosics: A Practical Approach*, Oxford University Press, Oxford; England, 1999). All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000). Enzymatic activity of HSD11B1 may be measured by *in vitro*, cell-based, or *in vivo* assays. Conveniently, HSD11B1 enzymatic activity can, for instance, be determined using a dehydrogenase and/or reductase activity assay.

In a preferred embodiment, the provided methods of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, or monitoring a treatment, or evaluating a treatment of a neurodegenerative disease further comprise comparing a level, or an activity, or both said level and said activity, of a transcription product of the gene coding for HSD11B1, and/or a translation product of the gene coding for HSD11B1, and/or a fragment, or derivative, or variant thereof in a series of samples taken from said subject over a period of time. In another preferred embodiment, said subject receives a treatment prior to one or more sample gatherings. It is a further preferred embodiment to determine said level, or said activity, or both said level and said activity, in said samples before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating a neurodegenerative disease, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:

at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the gene coding for HSD11B1, (ii) reagents that selectively detect a translation product of the gene coding for HSD11B1, (iii) reagents that selectively detect the presence or absence of a variation in the gene coding for HSD11B1; and

an instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop

such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the gene coding for HSD11B1, in a sample from said subject; and/or detecting the presence or absence of a variation in the gene coding for HSD11B1 in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status; or the presence of a variation in the gene coding for HSD11B1 indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease.

It is preferred that the reagents of the kit selectively detect the single nucleotide polymorphism located at chromosomal position 198633436 of human chromosome 1, located in the promoter region at position -2037 relative to the start codon of the gene coding for HSD11B1 (single nucleotide polymorphism identification number: rs846911). It is further preferred that said variation is a C to A transversion.

It is further preferred that the reagents of the kit selectively detect the single nucleotide polymorphism rs860185 located on human chromosome 1 in the promoter region at position -718 bases relative to the start codon of the gene coding for HSD11B1.

The kit according to the present invention may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD, in a subject. It is further useful in monitoring success or failure of therapeutic treatment of said subject.

In another aspect, the invention features a method of treating or preventing Alzheimer's disease or related neurodegenerative diseases, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the gene coding for HSD11B1, and/or (ii) a transcription product of the gene coding for HSD11B1, and/or (iii) a translation product of the gene coding for HSD11B1, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of the gene coding for HSD11B1, or a fragment, or derivative, or a variant thereof. An agent for treating or preventing a neurodegenerative disease, in particular AD, according to the instant invention, may also consist of a nucleotide, an oligonucleotide, or a polynucleotide. Said oligonucleotide or polynucleotide may comprise a nucleotide sequence of the HSD11B1 gene either in sense orientation or in antisense orientation.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy comprises several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26:274-278 and Mulligan, *Science*, 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane perturbation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, or electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3:743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells

(see e.g. Gillespie, *DN&P* 1992, 5:389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13:197-199; Crooke, *Biotechnology* 1992, 10:882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262:1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against the human gene coding for HSD11B1. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol*, 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy. A recently developed method of regulating the intracellular expression of genes by the use of double-stranded RNA, known variously as RNA interference (RNAi), can be another effective approach for nucleic acid therapy (Hannon, *Nature* 2002, 418: 244-251).

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection or liposomal mediated transfection (see McClland and Pardee, *Expression Genetics: Accelerated and High-Throughput Methods*, Eaton Publishing, Natick, MA, 1999).

In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular AD, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising

introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector. Said agent, particularly a therapeutic protein, can further be administered to said subject by a process comprising the injection or the systemic administration of a fusion protein, said fusion protein consisting of a fusion of a protein transduction domain with said agent.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for *in vitro* expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a fish, a fly, or a worm; a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neuro-degenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an Alzheimer's-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the

group consisting of (i) the gene coding for HSD11B1 and/or (ii) a transcription product of the gene coding for HSD11B1, and/or (iii) a translation product of the gene coding for HSD11B1, and/or (iv) a fragment, or derivative, or variant of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the gene coding for HSD11B1, and/or (ii) a transcription product of the gene coding for HSD11B1 and/or (iii) a translation product of the gene coding for HSD11B1, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the gene coding for HSD11B1, and/or (ii) a transcription product of the gene coding for HSD11B1, and/or (iii) a translation product of the gene coding for HSD11B1, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for a translation product of the HSD11B1 gene, or a fragment, or a derivative, or a variant thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v)

allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular Alzheimer's disease. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science*, 1989, 244:1288-1292 and Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1994 and Jackson and Abbott, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England, 1999). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease. Such an animal may be useful for screening, testing and validating compounds, agents and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) the gene coding for HSD11B1, and/or (ii) a transcription product of the gene coding for HSD11B1, and/or (iii) a translation product of the gene coding for HSD11B1, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the level, or the activity, or both the level and the activity of one or more substances recited in (i) to (iv), and (c) measuring the level, or the activity, or both the level and the activity of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the level and/or activity of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i)

the gene coding for HSD11B1, and/or (ii) a transcription product of the gene coding for HSD11B1, and/or (iii) a translation product of the gene coding for HSD11B1, and/or (iv) a fragment, or derivative, or variant of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the level and/or activity of one or more substances recited in (i) to (iv), and (c) measuring the level and/or activity of said substances in a matched control animal which is equally predisposed to developing or has already developed symptoms of said diseases and to which animal no such test compound has been administered, and (d) comparing the level and/or activity of the substance in the animals of step (b) and (c), wherein an alteration in the level and/or activity of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses the HSD11B1 gene, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional regulatory element which is not the native HSD11B1 gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the herein aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for a method of testing a compound, preferably an assay for screening a plurality of compounds, for inhibition of binding between a ligand and HSD11B1, or a fragment, or derivative, or variant thereof. Said method comprises the steps of (i) adding a liquid suspension of HSD11B1, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a compound, preferably a plurality of compounds, to be screened for said inhibition to said plurality of containers, and (iii) adding a detectable ligand, preferably a fluorescently detectable ligand, to said containers, and (iv) incubating the liquid suspension of HSD11B1, or said fragment, or derivative, or variant thereof, and said compounds, and said detectable, preferably said fluorescently detectable ligand, and (v) measuring the amounts of detectable

ligand or fluorescence associated with HSD11B1, or with said fragment, or derivative, or variant thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to HSD11B1, or said fragment, or derivative, or variant thereof. It might be preferred to reconstitute said HSD11B1 translation product, or fragment, or derivative, or variant thereof into artificial liposomes to generate the corresponding proteoliposomes to determine the inhibition of binding between a ligand and said HSD11B1 translation product. Methods of reconstitution of HSD11B1 translation products from detergent into liposomes have been detailed (Schwarz et al., *Biochemistry* 1999, 38: 9456-9464; Krivosheev and Usanov, *Biochemistry-Moscow* 1997, 62: 1064-1073). Instead of utilizing a fluorescently detectable label, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive label, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to HSD11B1, or a fragment, or derivative, or variant thereof. One example of a fluorescent binding assay, in this case based on the use of carrier particles, is disclosed and described in patent application WO 00/52451. A further example is the competitive assay method as described in patent WO 02/01226. Preferred signal detection methods for screening assays of the instant invention are described in the following patent applications: WO 96/13744, WO 98/16814, WO 98/23942, WO 99/17086, WO 99/34195, WO 00/66985, WO 01/59436, WO 01/59416.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and HSD11B1 by the herein aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In one further aspect, the invention features a method of testing a compound, preferably an assay for screening a plurality of compounds, to determine the degree of binding of said compound or compounds to HSD11B1, or to a fragment, or derivative, or variant thereof. Said method comprises the steps of (i) adding a liquid suspension of HSD11B1, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a detectable, preferably a fluorescently detectable compound, or adding a plurality of detectable, in particular fluorescently

detectable compounds, to be screened for said binding to said plurality of containers, and (iii) incubating the liquid suspension of HSD11B1, or said fragment, or derivative, or variant thereof, and said detectable compound, preferably said plurality of detectable compounds, and (iv) measuring the amounts of detectable compound or preferably of fluorescence associated with HSD11B1, or with said fragment, or derivative, or variant thereof, and (v) determining the degree of binding by one or more of said compounds to HSD11B1, or said fragment, or derivative, or variant thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a HSD11B1 gene product, or a fragment, or derivative, or variant thereof.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to HSD11B1 by the herein aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

The present invention features a protein molecule, said protein molecule being a translation product of the gene coding for HSD11B1, or a fragment, or derivative, or variant thereof, for the use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

The present invention further features a protein molecule, said protein molecule being a translation product of the gene coding for HSD11B1, or a fragment, or derivative, or variant thereof, for the use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the gene coding

for HSD11B1, or a fragment, or derivative, or variant thereof. The immunogen may comprise immunogenic or antigenic epitopes of portions of a translation product of said genes, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies*, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988). The term "antibody" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies as well as fragments thereof (see Dubel and Breitling, *Recombinant Antibodies*, Wiley-Liss, New York, NY, 1999). Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods, based on state-of-the-art techniques (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R., *Immunodiagnosics: A Practical Approach*, Oxford University Press, Oxford, England, 1999) such as enzyme-immuno assays (e.g. enzyme-linked immunosorbent assay, ELISA), radioimmuno assays, chemoluminescence-immuno assays, Western-blot, immunoprecipitation and antibody microarrays. These methods involve the detection of translation products of the HSD11B1 gene, or fragments, or derivatives, or variants thereof.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to Alzheimer's disease. Immuno-cytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the methods described in US patent 6150173.

Other features and advantages of the invention will be apparent from the following detailed description of the figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure legends:

Figure 1

Significance level p as a function of included SNPs in glucocorticoid-related genes and in APOE. Black bars indicate genes contributing to the disease risk significantly. A: entire sample ($n=776$), B: central European sample ($n= 391$), C: south European sample ($n=423$), D: entire sample excluding APOE.

Figure 2

Schematic representation of the promoter region of the gene coding for 11 β -hydroxysteroid dehydrogenase type 1 (HSD11B1) and of the analyzed SNPs. SNP rs846911 is located at -2037 relative to the start codon. SNP rs860185 is located at -718 . In contrast to the C allele, the A allele of rs846911 generates an OCT-1 binding site.

Figure 3

Diagram indicating the effects of the rare and the common haplotype containing SNPs rs846911 and rs860185 on the risk for the development of AD related to differential regulation of HSD11B1 transcription. The haplotype containing the rare variants of both SNPs (left bar) shows a significantly reduced luciferase activity (-20%) relative to the common variants containing haplotype (right bar).

Table 1

Genotyping results of 86 healthy controls and 77 AD patients for the SNP rs713073 located in the CAMK1G gene.

Table 2

Genotyping results of 79 healthy controls and 79 AD patients for the SNP rs926346 located in the IRF6 gene.

Example 1

Methods:

Genetic markers: Information on polymorphic sites was derived from the database of single nucleotide polymorphisms (dbSNP) established by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/SNP/index.html>). Twentyone SNP candidates in 10 genes were selected for genotyping. Of these SNP candidates, eleven were polymorphic in 100 chromosomes (rs915032 in NR5A1, rs6195 in NR3C1, rs2318376 in GMEB1, rs914563 in GMEB2, rs2272725 and rs877181 in NCOA2, rs948322 in MC2R, rs6159 in CRH, rs32897 in CRHBP, rs846911 in HSD11B1, and rs5479 in HSD11B2) and were genotyped in the study sample by the Masscode system (Kokoris et al., *Mol Diagn* 2000, 5:329-40). APOE genotyping was done on the LightCycler as described by Nauck et al. (*Clin Genet* 2000, 46:722-4).

Study sample: Genetic studies were conducted in 2 independent populations in central (n=391) and south Europe (n=423). The clinical diagnoses of AD (n=351) were made according to the NINCDS-ADRDA criteria, and were based on medical interview, physical examination, neuropsychological testing, brain MRI or CT, as well as blood and CSF tests. The mean onset age ($\pm$ standard deviation) of AD was 67 ± 9 years, the mean Mini-mental State Examination (MMSE) score was 20 ± 6 . There were 211 (60%) females among the AD patients. The control group (n=463) consisted of elderly individuals who were either the spouses of AD patients or subjects recruited from the outpatient clinics of the participating institutions. Dementia and memory deficits in control subjects were excluded by neuropsychological testing, consisting of the CERAD neuropsychological test battery and the MMSE. The mean age was 68 ± 9 years, the mean Mini-mental State Examination (MMSE) score was 29 ± 1 . There were 245 (53%) females among the control subjects. Informed consent was obtained from all participants, and the local human studies committees approved the study protocol.

Statistics: Analysis of genetic association with the disease was done by the recently developed set-association approach using the SUMSTAT program (<http://linkage.rockefeller.edu/ott/sumstat.html>). This method combines the information derived from measurements of allelic association and deviation from Hardy-Weinberg equilibrium into a single, genome-wide statistic. SNPs with high

Hardy-Weinberg disequilibrium (HWD) values in the control population are set equal to zero ('trimming'). For the remaining SNPs, effects of allelic association with disease and HWD values are combined into one statistic. Genome-wide significance of this statistic is calculated by permutation tests. In the present study, two SNPs with high HWD values ($\chi^2 \geq 3.9$) in the control population were trimmed. The number of permutation tests was set at 50000.

Results:

Genotyping of eleven SNPs in glucocorticoid-related genes and of *APOE* in 776 individuals revealed that two genes, *APOE* and *HSD11B1*, were associated with AD. The level of genome-wide significance reached by these two genes was equal to 0.002 (Figure 1A). The final corrected significance $p_{\min}$ was 0.006. Separate set-association analysis in the central and south European samples revealed similar results, i.e. *APOE* and *HSD11B1* contributed to AD risk (Figures 1B,C). In the south European sample (n=423), the gene encoding the adrenocorticotrophic hormone receptor (*MC2R*) was also associated with the disease. Set-association analysis excluding *APOE* confirmed that, among the examined genes, only *HSD11B1* is a susceptibility gene for AD (Figure 1D). The remaining genes failed to show, either separately or by interaction, significant contribution to the risk for the disease.

Conventional χ^2 analysis revealed significant overrepresentation ($p=0.008$) of the rare allele A of rs846911 in AD patients (2.9%) when compared with control subjects (0.5%). This difference corresponded to an odds ratio (OR) of 6.2 (95% confidence interval (CI): 1.4 - 28.4). The *APOE4* allele was also more frequent in AD patients (48.0%) than in control subjects (25.4%, $p<0.000001$), corresponding to an OR of 3.0 (95%CI: 2.1 - 3.5).

The single nucleotide polymorphism rs846911 is located in the promoter region of *HSD11B1*, 2037 bases 5' to the start codon. We also genotyped SNP rs860185 at -718 bases and observed that both SNPs were in complete linkage disequilibrium. In addition, the allelic distribution of rs860185 was identical to that of rs846911, indicating the presence of two haplotypes spanning 1.3 kb, the rare and disease-associated haplotype A-T and the frequent haplotype C-A. Computer assisted analysis (Quandt et al., *Nucleic Acids Res* 1995, 23:4878-84) of the *HSD11B1* promoter region showed that the rare allele A of rs846911 generates a new binding site for the octamer binding factor 1 (OCT-1) at -2037. The C allele failed to induce binding sites for transcription factors at this position (Figure 2).

Analysis of 11 polymorphisms in 10 glucocorticoid-related genes by applying the set-association approach in 776 AD patients and control subjects showed that a rare SNP of 11 β -HSD-1 (HSD11B1) is associated with a six-fold increased risk for AD. Nine genes failed to show, either separately or by interaction, significant contribution to the risk for the disease. In contrast to the marker-by-marker approach, the method used in this study was specifically developed for large case-control association studies with sets of polymorphic markers. By combining information derived from allelic association and from HWD, the set-association procedure is valid and powerful. Moreover, permutation tests provide a single, genome-wide statistic for association with disease (Hoh et al., *Genome Res* 2001, 11:2115-9).

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD-1, HSD11B1) by acting as an intracellular 11 β -reductase, regenerates biologically active hydrocortisone from inactive cortisone in neurons (Rajan et al., *J Neurosci* 1996, 16:65-70). This reductase activity would be anticipated to increase intraneuronal glucocorticoid levels, thus potentiating neurotoxicity. Indeed, 11-dehydrocorticosterone potentiates in vitro kainate-induced neurotoxicity in cultured hippocampal neurons, which is prevented by HSD11B1 inhibitors (Rajan et al., *supra*). Furthermore, lack of tissue glucocorticoid reactivation in HSD11B1 knockout mice reportedly ameliorates age-related learning impairment (Yau et al., *Proc Natl Acad Sci USA* 2001, 98:4716-21). The herein disclosed AD-associated SNP in HSD11B1 is located in the promoter region of the gene. Computer assisted analysis showed that the risk allele A generates a binding site for the octamer binding factor 1 (OCT-1). The random expectation value of the OCT-1 binding site is 4.8 matches per 1000 basepairs of DNA sequence. The C allele of rs846911 failed to induce binding sites for transcription factors at this position. It has been reported previously that OCT-1 binding sites generated by SNPs may lead to enhanced gene transcription (Knight et al., *Nat Genet* 1999, 22:145-50). Thus, the presence of the risk allele A may result in altered levels of transcription products of the gene coding for HSD11B1. To determine the effects of the haplotype block between the SNPs rs846911 and rs860185 on transcription regulation, we cloned haplotype-specific promoter fragments into the pGL3-Basic vector (Promega). Promoter activity was assayed using a dual-luciferase system (Promega) in human embryonic kidney cells (HEK-293T). Luciferase-containing constructs (pGL3) were co-transfected with pRL-TK synthetic renilla vector (Promega) to control for transfection efficiency. The Dual Luciferase system (Promega) was used according to the manufacturer's protocol, and experiments were repeated in twenty independent wells. Readings were taken

in duplicate on a luminometer. The risk-associated haplotype containing the rare variants of both SNPs reduced luciferase activity by -20% relative to the haplotype containing the common variants (Figure 3). This difference was highly significant. This result suggests that the effects of the haplotype containing SNPs rs846911 and rs860185 on risk for the development of AD are related to differential regulation of *HSD11B1* transcription. Reduced transcription activities, as a result of the rare risk haplotype may lead to low intracellular cortisol levels and thus to increased AD-associated inflammatory processes which promote neuronal death.

Set-association analysis showed that among the studied glucocorticoid-related genes only the gene coding for *HSD11B1* influenced the risk for AD significantly. Because we investigated one SNP per gene on average, the possibility still exists that unlinked causal genetic loci could have been missed. To exclude the possibility that the observed association is due to linkage disequilibrium (LD) of SNPs rs846911 and rs860185 with SNPs in the 5' and 3' adjacent genes of *HSD11B1*, we genotyped part of our population for SNP rs713073, which is located approximately 113 kb 5' upstream of *HSD11B1* in *CAMK1G* (encoding calcium/calmodulin-dependent protein kinase 1G) and for SNP rs926346 located approximately 84 kb 3' of *HSD11B1* in the gene *IRF6* (encoding interferon regulatory factor 6). Tables 1 and 2 show the lack of significant association of both SNPs, rs713073 and rs926346, respectively, with AD and suggest that the initially observed associations were not due to LD with SNPs in nearby genes.

To further exclude the possibility of LD with yet not identified SNPs in *HSD11B1* we sequenced in 24 chromosomes the 6 exons of *HSD11B1*, the intron-exon boundaries and the sequence up to 2.1 kb upstream the start codon. We found no evidence for additional coding SNPs or SNPs influencing splicing. Sequencing of 2.1 kb upstream the start codon revealed the absence of SNPs other than rs846911 and rs860185. Taken together, these results suggest that SNPs rs846911 and rs860185, which are in complete LD, are directly associated with the risk for AD. However, the median genomic length of the examined genes is 11.3 kb, thus the probability of linkage disequilibrium with the studied SNPs is very high.

Claims

1. A method for diagnosing or prognosticating a neurodegenerative disease in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, comprising detecting in a sample obtained from said subject the presence or absence of a variation in the gene coding for HSD11B1, wherein the presence of a variation in the gene coding for HSD11B1 in said subject indicates a diagnosis or prognosis of a neurodegenerative disease, or a measure for the propensity or predisposition to develop such a disease as compared to a subject who does not carry a variation in said gene.
2. The method according to claim 1 wherein said variation in the gene coding for HSD11B1 is a single nucleotide polymorphism located at chromosomal position 198633436 (single nucleotide polymorphism identification number: rs846911).
3. The method according to claims 1 and 2 wherein said variation is a C to A transversion.
4. The method according to any of claims 1 to 3 wherein said neurodegenerative disease is Alzheimer's disease.
5. A method for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of the gene coding for HSD11B1, or a translation product of gene coding for HSD11B1, or a fragment, or derivative, or variant thereof in a sample from said subject; and
comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in said subject, or determining the propensity or predisposition of said subject to develop such a disease.

6. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, said kit comprising:

at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the gene coding for HSD11B1, (ii) reagents that selectively detect a translation product of the gene coding for HSD11B1, (iii) reagents that selectively detect the presence or absence of a variation in the gene coding for HSD11B1; and

(b) an instruction for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the gene coding for HSD11B1, in a sample from said subject; and/or detecting the presence or absence of a variation in the gene coding for HSD11B1 in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status; or the presence of a variation in the gene coding for HSD11B1 indicates a diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

7. The kit according to claim 6, wherein said variation in the gene coding for HSD11B1 is a single nucleotide polymorphism located at chromosomal position 198633436 (single nucleotide polymorphism identification number: rs846911).

8. The kit according to claims 6 and 7, wherein said variation is a C to A transversion.

9. A protein molecule, said protein molecule being a translation product of the gene coding for HSD11B1, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

10. A protein molecule, said protein molecule being a translation product of the gene coding for HSD11B1, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

Figure 1

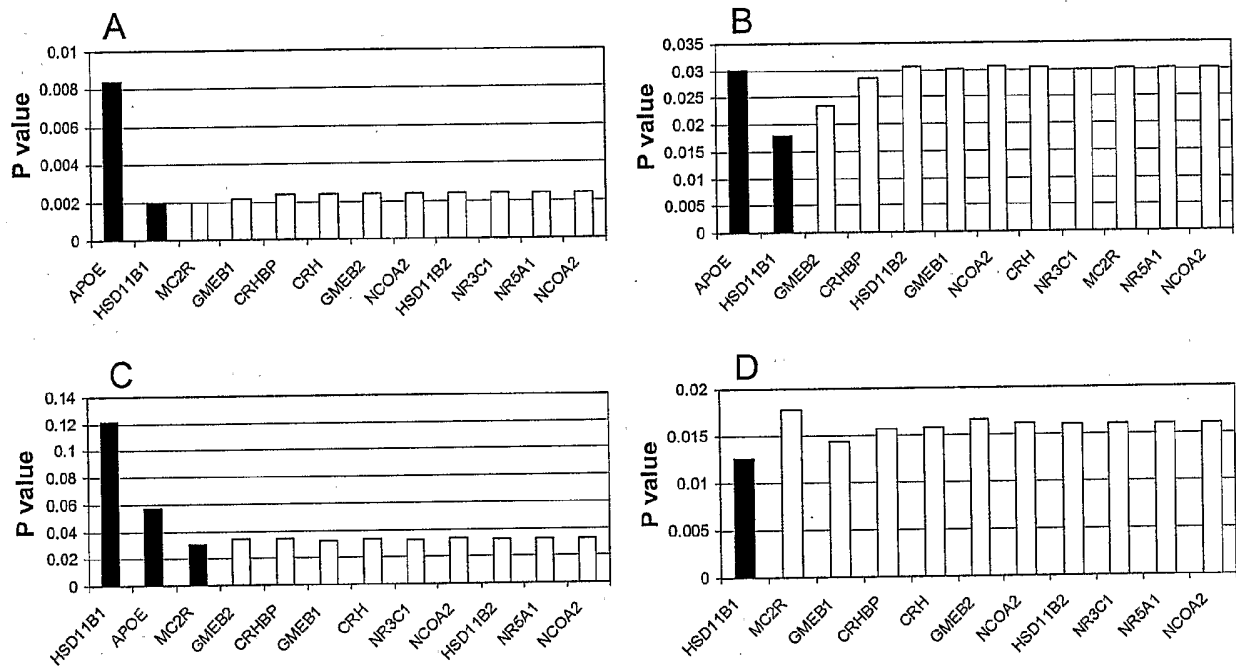


Figure 2

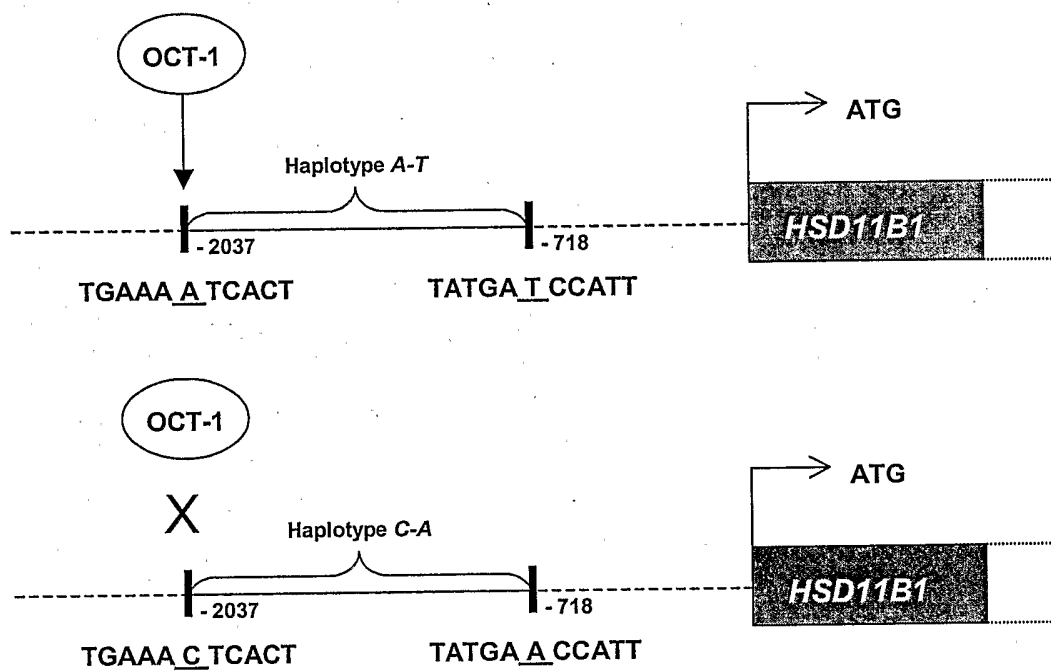
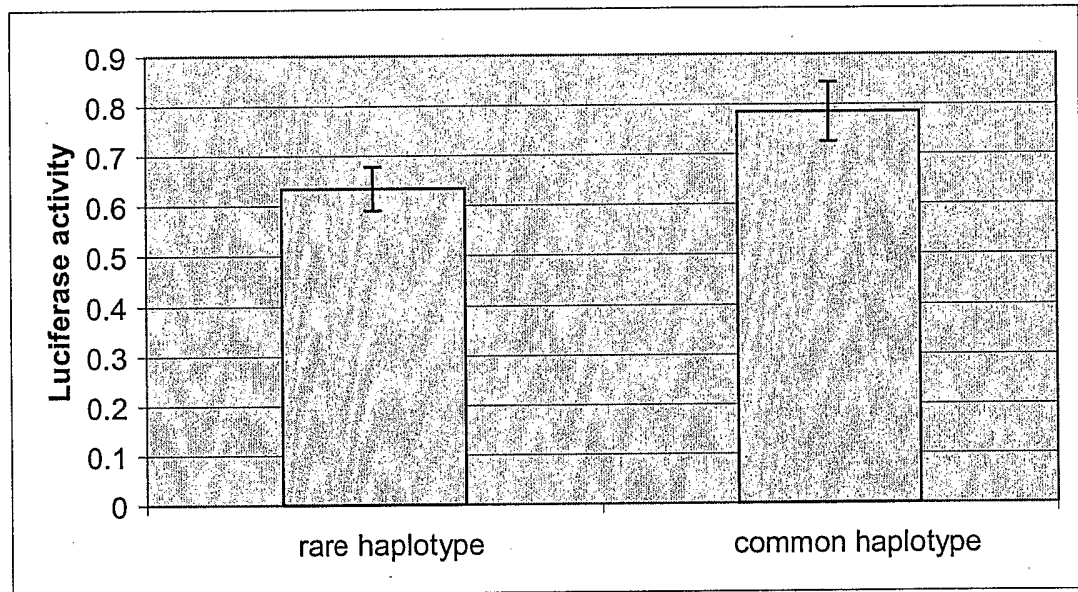


Figure 3

$p < 0.00001$

Table 1

			DIAGNOSIS		Total
			healthy controls	AD	
SNP rs713073	C/C	Number	43	33	76
		% of DIAGNOSIS	50.0%	42.9%	46.6%
	C/T	Number	34	35	69
		% of DIAGNOSIS	39.5%	45.5%	42.3%
	T/T	Number	9	9	18
		% of DIAGNOSIS	10.5%	11.7%	11.0%
Total	Number	86	77	163	
	% of DIAGNOSIS	100.0%	100.0%	100.0%	

p= 0.7

Table 2

			DIAGNOSIS		Total
			healthy controls	AD	
SNP rs926346	A/A	Number	51	54	105
		% of DIAGNOSIS	64.6%	68.4%	66.5%
	A/T	Number	28	22	50
		% of DIAGNOSIS	35.4%	27.8%	31.6%
	T/T	Number		3	3
		% of DIAGNOSIS		3.8%	1.9%
Total		Number	79	79	158
		% of DIAGNOSIS	100.0%	100.0%	100.0%

p=0.15