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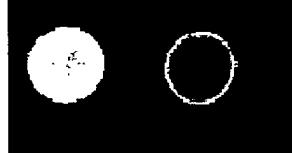
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(54) Title: DIAGNOSIS OF BEHAVIOURAL DISORDERS, NEUROLOGICAL DISORDERS AND CANCER

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(57) Abstract: The present invention relates to the chemically modified genomic sequences of genes associated with behavioural disorders, neurological disorders and cancer, to oligonucleotides and/or PNA-oligomers for detecting the cytosine methylation state of genes associated with behavioural disorders, neurological disorders and cancer which are directed against the sequence, as well as to a method for ascertaining genetic and/or epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer.

Diagnosis of behavioural disorders, neurological disorders and cancer**Field of the Invention**

The levels of observation that have been well studied by the methodological developments of recent years in molecular biology, are the genes themselves, the translation of these genes into RNA; and the resulting proteins. The question of which gene is switched on at which point in the course of the development of an individual, and how the activation and inhibition of specific genes in specific cells and tissues are controlled is correlatable to the degree and character of the methylation of the genes or of the genome. In this respect, pathogenic conditions may manifest themselves in a changed methylation pattern of individual genes or of the genome.

The present invention relates to nucleic acids, oligonucleotides, PNA-oligomers and to a method for the diagnosis and/or therapy of diseases which have a connection with the genetic and/or epigenetic parameters of genes associated with behaviour and, in particular, with the methylation status thereof.

Prior Art

Human behaviour is a developing system which is controlled in its early stage by genetic programming. External influences affect behaviour in utero and become more important after birth. Knowledge of natural and developmental influences as well as external determinants are necessary to understand behaviour in all. This understanding is the prerequisite for treating psychiatric disorders. Many aspects of behaviour are genetically controlled, indicated by twin studies. Usually, behavioural traits are complex and polygenically inherited, which requires elaborate analysis.

Included in behavioural disorders, which are associated with neurotransmitters, are major depressive disorder (Gurguis GN, Vo SP, Griffith JM, Rush AJ. Platelet alpha2A-adrenoceptor function in major depression: Gi coupling, effects of imipramine and relationship to treatment outcome. Psychiatry Res. 1999 Dec 20;89(2):73-95), schizophrenia Klimek V, Rajkowska G, Luker SN, Dilley G, Meltzer HY, Overholser JC, Stockmeier CA, Ordway GA. Brain noradrenergic receptors in major depression and schizophrenia Neuropsychopharmacology.

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1999 Jul;21(1):69-81) or Tourette syndrome (Comings DE, Gade-Andavolu R, Gonzalez N, Blake H, Wu S, MacMurray JP. Additive effect of three noradrenergic genes (ADRA2a, ADRA2C, DBH) on attention-deficit hyperactivity disorder and learning disabilities in Tourette syndrome subjects. Clin Genet. 1999 Mar;55(3):160-72). Neurotransmitters like dopamine and its receptors are associated with psychiatric and neurological disorders (Noble EP. The DRD2 gene in psychiatric and neurological disorders and its phenotypes. Pharmacogenomics. 2000 Aug;1(3):309-33). Studies in the past have shown that for example the dopamine D2 receptor gene is associated with alcoholism (Lu RB, Lee JF, Ko HC, Lin WW. Dopamine D2 receptor gene (DRD2) is associated with alcoholism with conduct disorder. Alcohol Clin Exp Res. 2001 Feb;25(2):177-84), personality traits like schizophrenia (Mowry BJ, Nancarrow DJ. Molecular genetics of schizophrenia. Clin Exp Pharmacol Physiol. 2001 Jan-Feb;28(1-2):66-9), drug abuse (Blomqvist O, Gelernter J, Kranzler HR. Family-based study of DRD2 alleles in alcohol and drug dependence. Am J Med Genet. 2000 Oct 9;96(5):659-64), smoking (Yoshida K, Hamajima N, Kozaki Ki, Saito H, Maeno K, Sugiura T, Ookuma K, Takahashi T. Association between the Dopamine D2 Receptor A2/A2 Genotype and Smoking behaviour in the Japanese. Cancer Epidemiol Biomarkers Prev. 2001 Apr;10(4):403-5) or compulsive gambling (Comings DE, Rosenthal RJ, Lesieur HR, Rugle LJ, Muhleman D, Chiu C, Dietz G, Gade R. A study of the dopamine D2 receptor gene in pathological gambling. Pharmacogenetics. 1996 Jun;6(3):223-34., and several personality traits). Dopamine associated disorders include furthermore human immunodeficiency virus dementia (Berger JR, Arendt G. HIV dementia: the role of the basal ganglia and dopaminergic systems. J Psychopharmacol. 2000;14(3):214-21) or migraine Lea RA, Dohy A, Jordan K, Quinlan S, Brimage PJ, Griffiths LR. Evidence for allelic association of the dopamine beta-hydroxylase gene (DBH) with susceptibility to typical migraine. Neurogenetics. 2000 Sep;3(1):35-40). behaviours in schizophrenic and schizoaffective patient are also associated with catechol-O-methyltransferase (Nolan KA, Volavka J, Czobor P, Cseh A, Lachman H, Saito T, Tiihonen J, Putkonen A, Hallikainen T, Kotilainen I, Rasanen P, Isohanni M, Jarvelin MR, Karvonen MK. Suicidal behaviour in patients with schizophrenia is related to COMT polymorphism. Psychiatr Genet. 2000 Sep;10(3):117-24).

Furthermore, it is increasingly being shown that some genes associated with behaviour, including those mentioned above have a wider role in the development of other diseases such as neurological disorders and cancers. For example, the dopamine receptor discussed above, in

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addition to being a key neurotransmitter involved in the regulation of the secretion of several anterior pituitary hormones, cardiovascular, and renal functions, has also been linked to the development of cancer (Role of dopamine in malignant tumor growth Basu S, Dasgupta PS Endocrine. 2000 Jun;12(3):237-41).

5-methylcytosine is the most frequent covalent base modification in the DNA of eukaryotic cells. It plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis.

Aberrant DNA methylation within CpG islands is common in human malignancies leading to abrogation or overexpression of a broad spectrum of genes (Jones, P.A. Cancer Res 65:2463-2467, 1996). Abnormal methylation has also been shown to occur in CpG rich regulatory elements in intronic and coding parts of genes for certain tumours (Chan, M.F., et al., Curr Top Microbiol Immunol 249:75-86,2000). Using restriction landmark genomic scanning, Costello and coworkers were able to show that methylation patterns are tumour-type specific (Costello, J. F., et al., Nat Genet 24:132-138, 2000). Highly characteristic DNA methylation patterns could also be shown for breast cancer cell lines (Huang, T. H.-M., et al., Hum Mol Genet 8:459-470, 1999). Genome wide assessment of methylation status represents a molecular fingerprint of cancer tissues.

Therefore, the identification of 5-methylcytosine as a component of genetic information is of considerable interest. However, 5-methylcytosine positions cannot be identified by sequencing since 5-methylcytosine has the same base pairing behaviour as cytosine. Moreover, the epigenetic information carried by 5-methylcytosine is completely lost during PCR amplification.

A relatively new and currently the most frequently used method for analyzing DNA for 5-methylcytosine is based upon the specific reaction of bisulfite with cytosine which, upon subsequent alkaline hydrolysis, is converted to uracil which corresponds to thymidine in its base pairing behaviour. However, 5-methylcytosine remains unmodified under these conditions. Consequently, the original DNA is converted in such a manner that methylcytosine, which originally could not be distinguished from cytosine by its hybridization behaviour, can now be detected as the only remaining cytosine using "normal" molecular biological techniques, for

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example, by amplification and hybridization or sequencing. All of these techniques are based on base pairing which can now be fully exploited. In terms of sensitivity, the prior art is defined by a method which encloses the DNA to be analyzed in an agarose matrix, thus preventing the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and which replaces all precipitation and purification steps with fast dialysis (Olek A, Oswald J, Walter J. A modified and improved method for bisulphite based cytosine methylation analysis. Nucleic Acids Res. 1996 Dec 15;24(24):5064-6). Using this method, it is possible to analyze individual cells, which illustrates the potential of the method. However, currently only individual regions of a length of up to approximately 3000 base pairs are analyzed, a global analysis of cells for thousands of possible methylation events is not possible. However, this method cannot reliably analyze very small fragments from small sample quantities either. These are lost through the matrix in spite of the diffusion protection.

An overview of the further known methods of detecting 5-methylcytosine may be gathered from the following review article: Rein, T., DePamphilis, M. L., Zorbas, H., Nucleic Acids Res. 1998, 26, 2255.

To date, barring few exceptions (e.g., Zeschnigk M, Lich C, Buiting K, Doerfler W, Horsthemke B. A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the SNRPN locus. Eur J Hum Genet. 1997 Mar-Apr;5(2):94-8) the bisulfite technique is only used in research. Always, however, short, specific fragments of a known gene are amplified subsequent to a bisulfite treatment and either completely sequenced (Olek A, Walter J. The pre-implantation ontogeny of the H19 methylation imprint. Nat Genet. 1997 Nov;17(3):275-6) or individual cytosine positions are detected by a primer extension reaction (Gonzalgo ML, Jones PA. Rapid quantitation of methylation differences at specific sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE). Nucleic Acids Res. 1997 Jun 15;25(12):2529-31, WO 95/00669) or by enzymatic digestion (Xiong Z, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. Nucleic Acids Res. 1997 Jun 15;25(12):2532-4). In addition, detection by hybridization has also been described (Olek et al., WO 99/28498).

Further publications dealing with the use of the bisulfite technique for methylation detection in individual genes are: Grigg G, Clark S. Sequencing 5-methylcytosine residues in genomic

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DNA. Bioessays. 1994 Jun;16(6):431-6, 431; Zeschnigk M, Schmitz B, Dittrich B, Buiting K, Horsthemke B, Doerfler W. Imprinted segments in the human genome: different DNA methylation patterns in the Prader-Willi/Angelman syndrome region as determined by the genomic sequencing method. Hum Mol Genet. 1997 Mar;6(3):387-95; Feil R, Charlton J, Bird AP, Walter J, Reik W. Methylation analysis on individual chromosomes: improved protocol for bisulphite genomic sequencing. Nucleic Acids Res. 1994 Feb 25;22(4):695-6; Martin V, Ribieras S, Song-Wang X, Rio MC, Dante R. Genomic sequencing indicates a correlation between DNA hypomethylation in the 5' region of the pS2 gene and its expression in human breast cancer cell lines. Gene. 1995 May 19;157(1-2):261-4; WO 97/46705, WO 95/15373 and WO 97/45560.

An overview of the Prior Art in oligomer array manufacturing can be gathered from a special edition of Nature Genetics (Nature Genetics Supplement, Volume 21, January 1999), published in January 1999, and from the literature cited therein.

Fluorescently labeled probes are often used for the scanning of immobilized DNA arrays. The simple attachment of Cy3 and Cy5 dyes to the 5'-OH of the specific probe are particularly suitable for fluorescence labels. The detection of the fluorescence of the hybridized probes may be carried out, for example via a confocal microscope. Cy3 and Cy5 dyes, besides many others, are commercially available.

Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem. 1988 Oct 15;60(20):2299-301). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapor phase in an unfragmented manner. The analyte is ionized by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones.

MALDI-TOF spectrometry is excellently suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut I G, Beck S. DNA and Matrix As-

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sisted Laser Desorption Ionization Mass Spectrometry. Current Innovations and Future Trends. 1995, 1; 147-57). The sensitivity to nucleic acids is approximately 100 times worse than to peptides and decreases disproportionately with increasing fragment size. For nucleic acids having a multiply negatively charged backbone, the ionization process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For the desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallization. There are now several responsive matrixes for DNA, however, the difference in sensitivity has not been reduced. The difference in sensitivity can be reduced by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. Phosphorothioate nucleic acids in which the usual phosphates of the backbone are substituted with thiophosphates can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut IG, Beck S. A procedure for selective DNA alkylation and detection by mass spectrometry. Nucleic Acids Res. 1995 Apr 25;23(8):1367-73). The coupling of a charge tag to this modified DNA results in an increase in sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities which make the detection of unmodified substrates considerably more difficult.

Genomic DNA is obtained from DNA of cell, tissue or other test samples using standard methods. This standard methodology is found in references such as Fritsch and Maniatis eds., Molecular Cloning: A Laboratory Manual, 1989.

Description

The object of the present invention is to provide the chemically modified DNA of genes associated with behavioural disorders, neurological disorders and cancer, as well as oligonucleotides and/or PNA-oligomers for detecting cytosine methylations, as well as a method which is particularly suitable for the diagnosis and/or therapy of genetic and epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer. The present invention is based on the discovery that genetic and epigenetic parameters and, in particular, the cytosine methylation pattern of genes associated with behavioural disorders, neurological disorders and cancer are particularly suitable for the diagnosis and/or therapy of behavioural disorders, neurological disorders and cancer.

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This objective is achieved according to the present invention using a nucleic acid containing a sequence of at least 18 bases in length of the chemically pretreated DNA of genes associated with behavioural disorders, neurological disorders and cancer according to one of Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto.

The chemically modified nucleic acid could heretofore not be connected with the ascertainment of genetic and epigenetic parameters.

The object of the present invention is further achieved by an oligonucleotide or oligomer for detecting the cytosine methylation state in chemically pretreated DNA, containing at least one base sequence having a length of at least 13 nucleotides which hybridizes to a chemically pretreated DNA of genes associated with behavioural disorders, neurological disorders and cancer according to Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto. The oligomer probes according to the present invention constitute important and effective tools which, for the first time, make it possible to ascertain the genetic and epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer. The base sequence of the oligomers preferably contains at least one CpG dinucleotide. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Particularly preferred are oligonucleotides according to the present invention in which the cytosine of the CpG dinucleotide is the 5th - 9th nucleotide from the 5'-end of the 13-mer; in the case of PNA-oligomers, it is preferred for the cytosine of the CpG dinucleotide to be the 4th - 6th nucleotide from the 5'-end of the 9-mer.

The oligomers according to the present invention are normally used in so called "sets" which contain at least one oligomer for each of the CpG dinucleotides of the sequences of Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto. Preferred is a set which contains at least one oligomer for each of the CpG dinucleotides from one of Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto.

Moreover, the present invention makes available a set of at least two oligonucleotides which can be used as so-called "primer oligonucleotides" for amplifying DNA sequences of one of Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto, or segments thereof.

In the case of the sets of oligonucleotides according to the present invention, it is preferred that at least one oligonucleotide is bound to a solid phase. It is further preferred that all the oligonucleotides of one set are bound to a solid phase.

The present invention moreover relates to a set of at least 10 n (oligonucleotides and/or PNA-oligomers) used for detecting the cytosine methylation state in chemically pretreated genomic DNA (Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto). These probes enable diagnosis and/or therapy of genetic and epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer. The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) in the chemically pre-treated DNA of genes associated with behavioural disorders, neurological disorders and cancer according to one of Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto.

According to the present invention, it is preferred that an arrangement of different oligonucleotides and/or PNA-oligomers (a so-called "array") made available by the present invention is present in a manner that it is likewise bound to a solid phase. This array of different oligonucleotide- and/or PNA-oligomer sequences can be characterized in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid phase surface is preferably composed of silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver, or gold. However, nitrocellulose as well as plastics such as nylon which can exist in the form of pellets or also as resin matrices are possible as well.

Therefore, a further subject matter of the present invention is a method for manufacturing an array fixed to a carrier material for analysis in connection with behavioural disorders, neurological disorders and cancer in which method at least one oligomer according to the present invention is coupled to a solid phase. Methods for manufacturing such arrays are known, for example, from US Patent 5,744,305 by means of solid-phase chemistry and photolabile protecting groups.

A further subject matter of the present invention relates to a DNA chip for the analysis of behavioural disorders, neurological disorders and cancer which contains at least one nucleic acid

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according to the present invention. DNA chips are known, for example, for US Patent 5,837,832.

Moreover, a subject matter of the present invention is a kit which may be composed, for example, of a bisulfite-containing reagent, a set of primer oligonucleotides containing at least two oligonucleotides whose sequences in each case correspond or are complementary to an 18 base long segment of the base sequences specified in the appendix (Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto), oligonucleotides and/or PNA-oligomers as well as instructions for carrying out and evaluating the described method. However, a kit along the lines of the present invention can also contain only part of the aforementioned components.

The present invention also makes available a method for ascertaining genetic and/or epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer by analyzing cytosine methylations and single nucleotide polymorphisms, including the following steps:

In the first step of the method, a genomic DNA sample is chemically treated in such a manner that cytosine bases which are unmethylated at the 5'-position are converted to uracil, thymine, or another base which is dissimilar to cytosine in terms of hybridization behaviour. This will be understood as 'chemical pretreatment' hereinafter.

The genomic DNA to be analyzed is preferably obtained from usual sources of DNA such as cells or cell components, for example, cell lines, biopsies, blood, sputum, stool, urine, cerebro-spinal fluid, tissue embedded in paraffin such as tissue from eyes, intestine, kidney, brain, heart, prostate, lung, breast or liver, histologic object slides, or combinations thereof.

The above described treatment of genomic DNA is preferably carried out with bisulfite (hydrogen sulfite, disulfite) and subsequent alkaline hydrolysis which results in a conversion of non-methylated cytosine nucleobases to uracil or to another base which is dissimilar to cytosine in terms of base pairing behaviour.

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Fragments of the chemically pretreated DNA are amplified, using sets of primer oligonucleotides according to the present invention, and a, preferably heat-stable polymerase. Because of statistical and practical considerations, preferably more than ten different fragments having a length of 100 - 2000 base pairs are amplified. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel. Usually, the amplification is carried out by means of a polymerase chain reaction (PCR).

In a preferred embodiment of the method, the set of primer oligonucleotides includes at least two oligonucleotides whose sequences are each reverse complementary or identical to an at least 18 base-pair long segment of the base sequences specified in the appendix (Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto). The primer oligonucleotides are preferably characterized in that they do not contain any CpG dinucleotides.

According to the present invention, it is preferred that at least one primer oligonucleotide is bonded to a solid phase during amplification. The different oligonucleotide and/or PNA-oligomer sequences can be arranged on a plane solid phase in the form of a rectangular or hexagonal lattice, the solid phase surface preferably being composed of silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver, or gold, it being possible for other materials such as nitrocellulose or plastics to be used as well.

The fragments obtained by means of the amplification can carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer, it being preferred that the fragments that are produced have a single positive or negative net charge for better detectability in the mass spectrometer. The detection may be carried out and visualized by means of matrix assisted laser desorption/ionization mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

The amplificates obtained in the second step of the method are subsequently hybridized to an array or a set of oligonucleotides and/or PNA probes. In this context, the hybridization takes place in the manner described in the following. The set of probes used during the hybridization is preferably composed of at least 10 oligonucleotides or PNA-oligomers. In the process, the amplificates serve as probes which hybridize to oligonucleotides previously bonded to a

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solid phase. The non-hybridized fragments are subsequently removed. Said oligonucleotides contain at least one base sequence having a length of 13 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in the appendix, the segment containing at least one CpG dinucleotide. The cytosine of the CpG dinucleotide is the 5th to 9th nucleotide from the 5'-end of the 13-mer. One oligonucleotide exists for each CpG dinucleotide. Said PNA-oligomers contain at least one base sequence having a length of 9 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in the appendix, the segment containing at least one CpG dinucleotide. The cytosine of the CpG dinucleotide is the 4th to 6th nucleotide seen from the 5'-end of the 9-mer. One oligonucleotide exists for each CpG dinucleotide.

In the fourth step of the method, the non-hybridized amplificates are removed.

In the final step of the method, the hybridized amplificates are detected. In this context, it is preferred that labels attached to the amplificates are identifiable at each position of the solid phase at which an oligonucleotide sequence is located.

According to the present invention, it is preferred that the labels of the amplificates are fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer. The mass spectrometer is preferred for the detection of the amplificates, fragments of the amplificates or of probes which are complementary to the amplificates, it being possible for the detection to be carried out and visualized by means of matrix assisted laser desorption/ionization mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

The produced fragments may have a single positive or negative net charge for better detectability in the mass spectrometer. The aforementioned method is preferably used for ascertaining genetic and/or epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer.

The oligomers according to the present invention or arrays thereof as well as a kit according to the present invention are intended to be used for the diagnosis and/or therapy of behavioural disorders, neurological disorders and cancer by analyzing methylation patterns of genes

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associated with behavioural disorders, neurological disorders and cancer. According to the present invention, the method is preferably used for the diagnosis and/or therapy of important genetic and/or epigenetic parameters within genes associated with behavioural disorders, neurological disorders and cancer.

The method according to the present invention is used, for example, for the diagnosis and/or therapy of behavioural disorders, neurological disorders and cancer.

The nucleic acids according to the present invention of Seq. ID No.1 through Seq. ID No.46 and sequences complementary thereto can be used for the diagnosis and/or therapy of genetic and/or epigenetic parameters of genes associated with behavioural disorders, neurological disorders and cancer.

The present invention moreover relates to a method for manufacturing a diagnostic agent and/or therapeutic agent for the diagnosis and/or therapy of behavioural disorders, neurological disorders and cancer by analyzing methylation patterns of genes associated with behavioural disorders, neurological disorders and cancer, the diagnostic agent and/or therapeutic agent being characterized in that at least one nucleic acid according to the present invention is used for manufacturing it, possibly together with suitable additives and auxiliary agents.

A further subject matter of the present invention relates to a diagnostic agent and/or therapeutic agent for behavioural disorders, neurological disorders and cancer by analyzing methylation patterns of genes associated with behavioural disorders, neurological disorders and cancer, the diagnostic agent and/or therapeutic agent containing at least one nucleic acid according to the present invention, possibly together with suitable additives and auxiliary agents.

The present invention moreover relates to the diagnosis and/or prognosis of events which are disadvantageous to patients or individuals in which important genetic and/or epigenetic parameters within genes associated with behavioural disorders, neurological disorders and cancer said parameters obtained by means of the present invention may be compared to another set of genetic and/or epigenetic parameters, the differences serving as the basis for a diagnosis and/or prognosis of events which are disadvantageous to patients or individuals.

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In the context of the present invention the term "hybridization" is to be understood as a bond of an oligonucleotide to a completely complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure. To be understood by "stringent hybridization conditions" are those conditions in which a hybridization is carried out at 60°C in 2.5 x SSC buffer, followed by several washing steps at 37°C in a low buffer concentration, and remains stable.

The term "functional variants" denotes all DNA sequences which are complementary to a DNA sequence, and which hybridize to the reference sequence under stringent conditions and have an activity similar to the corresponding polypeptide according to the present invention.

In the context of the present invention, "genetic parameters" are mutations and polymorphisms of genes associated with behavioural disorders, neurological disorders and cancer and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms).

In the context of the present invention, "epigenetic parameters" are, in particular, cytosine methylations and further chemical modifications of DNA bases of genes associated with behavioural disorders, neurological disorders and cancer and sequences further required for their regulation. Further epigenetic parameters include, for example, the acetylation of histones which, however, cannot be directly analyzed using the described method but which, in turn, correlates with the DNA methylation.

In the following, the present invention will be explained in greater detail on the basis of the sequences and examples with reference to the accompanying drawing without being limited thereto.

Figure 1

Figure 1 shows the hybridisation of fluorescent labelled amplificates to a surface bound oligonucleotide. Fluorescence at a spot shows hybridisation of the amplificate to the oligonucleotide. Hybridisation to a CG oligonucleotide denotes methylation at the cytosine position being analysed, hybridisation to a TG oligonucleotide denotes no methylation at the

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cytosine position being analysed. It can be seen that Sample II had a higher degree of methylation than Sample I.

Seq ID No.1 trough Seq ID 46

Sequences having odd sequence numbers (e.g., Seq. ID No. 1, 3, 5, ...) exhibit in each case sequences of the chemically pretreated genomic DNAs of different genes associated with behavioural disorders, neurological disorders and cancer. Sequences having even sequence numbers (e.g., Seq. ID No. 2, 4, 6, ...) exhibit in each case the sequences of chemically pretreated genomic DNAs. Said genomic DNAs are complementary to the genomic DNAs from which the preceding sequence was derived (e.g., the complementary sequence to the genomic DNA from which Seq. ID No.1 is derived is the genomic sequence from which Seq. ID No.2 is derived, the complementary sequence to the genomic DNA from which Seq. ID No.3 is derived is the sequence from which Seq. ID No.4 is derived, etc.).

Seq ID No.47 trough Seq ID 50

Seq ID No.47 trough Seq ID 50 show the sequences of oligonucleotides used in Example 1

Example 1 :Methylation analysis of the angiotensin gene

The following example relates to a fragment of the angiotensin gene in which a specific CG-position is to be analyzed for methylation.

In the first step, a genomic sequence is treated using bisulfite (hydrogen sulfite, disulfite) in such a manner that all cytosines which are not methylated at the 5-position of the base are modified in such a manner that a different base is substituted with regard to the base pairing behavior while the cytosines methylated at the 5-position remain unchanged.

If bisulfite solution is used for the reaction, then an addition takes place at the non-methylated cytosine bases. Moreover, a denaturing reagent or solvent as well as a radical interceptor must be present. A subsequent alkaline hydrolysis then gives rise to the conversion of non-methylated cytosine nucleobases to uracil. The chemically converted DNA is then used for the detection of methylated cytosines. In the second method step, the treated DNA sample is diluted with water or an aqueous solution. Preferably, the DNA is subsequently desulfonated. In the third step of the method, the DNA sample is amplified in a polymerase chain reaction,

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preferably using a heat-resistant DNA polymerase. In the present case, cytosines of the angiotensin gene are analyzed. To this end, a defined fragment having a length of 507bp is amplified with the specific primer oligonucleotides TGAGYGGGTAGTAGGGTTAG (Sequence ID 47) and CRACTTACCTTCTACTATAA (Sequence ID No. 48). The single gene PCR reaction was performed on a thermocycler (Eppendorf GmbH) using bisulfite DNA 10 ng, primer 6 pmole each, dNTP 200 µM each, 1.5 mM MgCl₂ and 1 U HotstartTaq (Qiagen AG). The other conditions were as recommended by the Taq polymerase manufacturer. In the multiplex PCR up to 16 primer pairs were used within the PCR reaction. The multiplex PCR was done according the single gene PCR with the following modifications: primer 0.35 pmole each, dNTP 800 µM each and 4,5 mM MgCl₂. The cycle program for single gene PCR and multiplex PCR was as followed: step 1,14 min 96 °C; step 2, 60 sec 96°C; step 3, 45 sec 55 °C; step 4 ,75 sec 72 °C; step 5, 10 min 72 °C; the step 2 to step 4 were repeated 39 fold.

The amplificate serves as a sample which hybridizes to an oligonucleotide previously bound to a solid phase, forming a duplex structure, for example ATATTTCGGGGTTGGG (Sequence ID No. 49), the cytosine to be detected being located at position 119 of the amplificate. The detection of the hybridization product is based on Cy3 and Cy5 fluorescently labelled primer oligonucleotides which have been used for the amplification. A hybridization reaction of the amplified DNA with the oligonucleotide takes place only if a methylated cytosine was present at this location in the bisulfite-treated DNA. Thus, the methylation status of the specific cytosine to be analyzed is inferred from the hybridization product.

In order to verify the methylation status of the position, a sample of the amplificate is further hybridized to another oligonucleotide previously bonded to a solid phase. Said oligonucleotide is identical to the oligonucleotide previously used to analyze the methylation status of the sample, with the exception of the position in question. At the position to be analysed said oligonucleotide comprises a thymine base as opposed to a cytosine base i.e ATATTTTGCGGGTTGGG (Sequence ID No. 50). Therefore, the hybridisation reaction only takes place if an unmethylated cytosine was present at the position to be analysed.

Example 2: Diagnosis of behavioural disorders, neurological disorders and cancer

In order to relate the methylation patterns to one of the behavioural disorders, neurological disorders and cancer, it is initially required to analyze the DNA methylation patterns of a

group of diseased and of a group of healthy patients. These analyses are carried out, for example, analogously to Example 1. The results obtained in this manner are stored in a database and the CpG dinucleotides which are methylated differently between the two groups are identified. This can be carried out by determining individual CpG methylation rates as can be done, for example, in a relatively imprecise manner, by sequencing or else, in a very precise manner, by a methylation-sensitive "primer extension reaction". It is particularly preferred that the determination be carried out in the manner described in Example 1, bisulphite treatment of genomic DNA followed by fluorescence hybridisation analysis on an oligomer array, thereby enabling the simultaneous analysis of multiple positions within the genome. It is also possible for the entire methylation status to be analyzed simultaneously, and for the patterns to be compared, for example, by clustering analyses which can be carried out, for example, by a computer.

Subsequently, it is possible to allocate the examined patients to a specific therapy group and to treat these patients selectively with an individualized therapy. Example 2 can be carried out, for example, for behavioural disorders, neurological disorders and cancer, in particular major depressive disorder, schizophrenia, Tourette syndrome, psychiatric and neurological disorders, in particular alcoholism, personality traits, drug abuse, smoking, compulsive gambling, human immunodeficiency virus dementia, migraine, behaviours in schizophrenic and schizoaffective patients and suicidal behaviour in patients with schizophrenia.

Claims

1. A nucleic acid comprising a sequence at least 18 bases in length of a segment of the chemically pretreated DNA of genes associated with behavioural disorders, neurological disorders and cancer according to one of the sequences taken from the group of Seq. ID No.1 to Seq. ID No.46 and sequences complementary thereto.
2. A nucleic acid comprising a sequence at least 18 base pairs in length of a segment of the chemically pretreated DNA of genes associated with behavioural disorders, neurological disorders and cancer according to the gene adrenergic alpha-1C- receptor and sequences complementary thereto.
3. An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer comprising in each case at least one base sequence having a length of at least 9 nucleotides which hybridizes to or is identical to a chemically pretreated DNA of genes associated with behavioural disorders, neurological disorders and cancer according to one of the Seq ID Nos 1 to 46 according to claim 1 or to a chemically pretreated DNA of the gene according to claim 2 and sequences complementary thereto.
4. The oligomer as recited in Claim 3; wherein the base sequence includes at least one CpG dinucleotide
5. The oligomer as recited in Claim 3, characterized in that the cytosine of the CpG dinucleotide is located approximately in the middle third of the oligomer.
6. A set of oligomers, comprising at least two oligomers according to any of claims 3 to 5.
7. A set of oligomers as recited in Claim 6, comprising oligomers for detecting the methylation state of all CpG dinucleotides within one of the sequences according to Seq. ID Nos. 1 through 46 according to claim 1 or a chemically pretreated DNA of genes according to claim 2, and sequences complementary thereto.

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8. A set of at least two oligonucleotides as recited in Claim 3, which can be used as primer oligonucleotides for the amplification of DNA sequences of one of Seq. ID 1 through Seq. ID 46 and sequences complementary thereto and/or sequences of a chemically pretreated DNA of genes according to claim 2, and sequences complementary thereto and segments thereof.
9. A set of oligonucleotides as recited in Claim 8, characterized in that at least one oligonucleotide is bound to a solid phase.
10. Use of a set of oligomer probes comprising at least ten of the oligomers according to any of claims 6 through 9 for detecting the cytosine methylation state and/or single nucleotide polymorphisms (SNPs) in a chemically pretreated genomic DNA according to claim 1 or a chemically pretreated DNA of genes according to claim 2.
11. A method for manufacturing an arrangement of different oligomers (array) fixed to a carrier material for analyzing diseases associated with the methylation state of the CpG dinucleotides of one of the Seq. ID 1 through Seq. ID 46 and sequences complementary thereto and/or chemically pretreated DNA of genes according to claim 2, wherein at least one oligomer according to any of the claims 3 through 5 is coupled to a solid phase.
12. An arrangement of different oligomers (array) obtainable according to claim 11.
13. An array of different oligonucleotide- and/or PNA-oligomer sequences as recited in Claim 12, characterized in that these are arranged on a plane solid phase in the form of a rectangular or hexagonal lattice.
14. The array as recited in any of the Claims 12 or 13, characterized in that the solid phase surface is composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold.
15. A DNA- and/or PNA-array for analyzing diseases associated with the methylation state of genes, comprising at least one nucleic acid according to one of the preceding claims.

16. A method for ascertaining genetic and/or epigenetic parameters for the diagnosis and/or therapy of existing diseases or the predisposition to specific diseases by analyzing cytosine methylations, characterized in that the following steps are carried out:
 - in a genomic DNA sample, cytosine bases which are unmethylated at the 5-position are converted, by chemical treatment, to uracil or another base which is dissimilar to cytosine in terms of hybridization behaviour;
 - fragments of the chemically pretreated genomic DNA are amplified using sets of primer oligonucleotides according to Claim 8 or 9 and a polymerase, the amplificates carrying a detectable label;
 - amplificates are hybridized to a set of oligonucleotides and/or PNA probes according to the Claims 6 and 7, or else to an array according to one of the Claims 12 through 15;
 - the hybridized amplificates are subsequently detected.
17. The method as recited in Claim 16, characterized in that the chemical treatment is carried out by means of a solution of a bisulfite, hydrogen sulfite or disulfite.
18. The method as recited in one of the Claims 16 or 17, characterized in that more than ten different fragments having a length of 100 - 2000 base pairs are amplified.
19. The method as recited in one of the Claims 16 through 18, characterized in that the amplification of several DNA segments is carried out in one reaction vessel.
20. The method as recited in one of the Claims 16 through 19, characterized in that the polymerase is a heat-resistant DNA polymerase.
21. The method as recited in Claim 20, characterized in that the amplification is carried out by means of the polymerase chain reaction (PCR).
22. The method as recited in one of the Claims 16 through 21, characterized in that the labels of the amplificates are fluorescence labels.

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23. The method as recited in one of the Claims 16 through 21, characterized in that the labels of the amplificates are radionuclides.
24. The method as recited in one of the Claims 16 through 21, characterized in that the labels of the amplificates are detachable molecule fragments having a typical mass which are detected in a mass spectrometer.
25. The method as recited in one of the Claims 16 through 21, characterized in that the amplificates or fragments of the amplificates are detected in the mass spectrometer.
26. The method as recited in one of the Claims 24 and/or 25, characterized in that the produced fragments have a single positive or negative net charge for better detectability in the mass spectrometer.
27. The method as recited in one of the Claims 24 through 26, characterized in that detection is carried out and visualized by means of matrix assisted laser desorption/ionization mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).
28. The method as recited in one of the Claims 16 through 27, characterized in that the genomic DNA is obtained from cells or cellular components which contain DNA, sources of DNA comprising, for example, cell lines, biopsies, blood, sputum, stool, urine, cerebro-spinal fluid, tissue embedded in paraffin such as tissue from eyes, intestine, kidney, brain, heart, prostate, lung, breast or liver, histologic object slides, and all possible combinations thereof.
29. A kit comprising a bisulfite (= disulfite, hydrogen sulfite) reagent as well as oligonucleotides and/or PNA-oligomers according to one of the Claims 3 through 5.
30. The use of a nucleic acid according to Claims 1 or 2, of an oligonucleotide or PNA-oligomer according to one of the Claims 3 through 5, of a kit according to Claim 29, of an array according to one of the Claims 12 through 15, of a set of oligonucleotides according to one of claims 6 through 9 for the diagnosis of behavioural disorders, neurological disorders and cancer, , in particular major depressive disorder, schizophrenia,

Tourette syndrome, psychiatric and neurological disorders, in particular alcoholism, personality traits, drug abuse, smoking, compulsive gambling, human immunodeficiency virus dementia, migraine, behaviours in schizophrenic and schizoaffective patients and suicidal behaviour in patients with schizophrenia.

31. The use of a nucleic acid according to Claims 1 or 2, of an oligonucleotide or PNA-oligomer according to one of Claims 3 through 5, of a kit according to Claim 29, of an array according to one of the Claims 12 through 15, of a set of oligonucleotides according to one of claims 6 through 9 for the therapy of behavioural disorders, neurological disorders and cancer , in particular major depressive disorder, schizophrenia, Tourette syndrome, psychiatric and neurological disorders, in particular alcoholism, personality traits, drug abuse, smoking, compulsive gambling, human immunodeficiency virus dementia, migraine, behaviours in schizophrenic and schizoaffective patients and suicidal behaviour in patients with schizophrenia.

Fig. 1

1/1



Sequence listing

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<221> unsure

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<211> 6123

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 29

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<211> 6123

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (*Homo sapiens*)

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<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<221> unsure

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<211> 4850

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 35

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<210> 36

<211> 4850

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2375

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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cggatcgccgg	tccgggtcggg	tcgagcgtcg	agcggatgg	agcggccggcg	gcggccggcg	2220
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ttagtttattt	gtgggtttagt	gagaagagta	aggttacgtc	gtcgcgcgc	gttagtaaga	2340
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<210> 38
<211> 2375

<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)

<400> 38

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tggtcgtat ttcgggtttc gatgttggac ggagattcgg gtttggaaata cgataggat
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tttgatgtag	gttttgatta	gggatgttt	ttttaaattt	agatttgatg	gagttattgt	2040
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gaggggttt	taggatttat	tatattttgt	ttaaagtaga	ttaggtgaat	aaattnntta	2160
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tttttgtat	ttggagttt	tttattaatt	tatttattt	tttttatttt	tgttttttg	2280
ttgtttttt	ggttttgaag	ttttttgtg	tattttttt	cgtggataat	tttattttat	2340
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<210> 39

<211> 2037

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 39

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tttttaagg	tttatttagag	attnnttaag	agtgtttttt	ttttggttt	ggagataatt	180
ttattnatatt	gttattatitt	attgtatagg	tgattatgt	tttggttttt	atttgtttgg	240
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ttgagtttgg	tttttagga	tggttgggtt	attgtgggt	agagagtatt	tttgcgagg	360
gttaggggaa	ggttggatt	tttttaaat	tttacgttgc	tgtaaagata	tagttatttt	420
ttttatagc	gtagagtta	gagttgaagt	agttttttaga	tttggtttta	tgggtattta	480
aggattnaaa	tgttgggtt	atgtttatgt	atgaatgtgt	ttttaagtt	agattnnnggg	540
ttttttaga	gagacgagaa	ataggaggaa	aaaggaagga	agggagggag	gagttggggg	600
aggagggata	gttttttagt	tagttggaga	gattgtttaa	tttagtttgg	gggttggagt	660
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ggagtaggtt	tgttatttt	ttttttttt	ttaaaggtt	ttattnnttag	tagtgttattt	960
tgtagtttgg	ttattnntt	tgtagtttta	tttcgtttt	tttttagtaa	tgtattnntac	1020
gtttatgttt	tttattttt	atgtgtttt	cgcgttttt	ggagtagtag	tggatttagt	1080
tggaggttgg	ggaaaaattt	agaggagata	aaagtggaa	gagtaggtcg	tggagagagt	1140
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ggcgtaagtt	tgtgggaggg	aaaatttggat	tttctgttag	ataaaatgt	ttattcgtgt	1620
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tttttggagt	ttttatggaa	tgttagttat	attnaggagg	ttattnattt	ttagtttttg	1920
gtgcggaggt	ttaaggttgg	cgttttggtt	gggatgttgc	atcgtggcga	gtttgagaac	1980
gtagatttcg	tggtggttt	gatcgatggg	gatattgttt	atttgcgggt	gagttttt	2037

<210> 40

<211> 2037

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 40

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ttaagttcg	ttacggtcgg	atatttaaa	taggacgtt	gttttgagtt	ttcgttattag	120
gagttggaaa	tggatggttt	tttgggtgta	gttgatattt	tatgagagtt	ttagggattt	180
tttcgggtt	agggggatgt	gataggggag	ggggtttta	cggggagtgc	agttttgtag	240
tgccgttatt	aggatgatta	ggaagatgtt	tattgttgc	ttgtatatga	aggttggttt	300
tcgtatgtt	gggtcgggt	ggttgttta	gcgattgagg	gcgggtatgg	ttggggtgag	360
ttttttggtt	ttatgttgc	attatgttt	tggttttga	tggggtggtt	ttaggtagta	420
cgggttaatt	tattgttta	gcgggaaatt	taatttttt	ttttataagt	ttgcgttta	480
ggttaagtat	ttagttgtt	gtttttttt	aattggtatt	aatgatata	ggacgttatt	540
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tttaaaagta	gatgtatttt	gtagttaa	gtttttttt	atattttt	gttaggtatt	660
agttgattgt	ttttaggtt	gtttgttta	gttttttagt	ggatgggtt	tgcgtcgtt	720
tttatttacgg	atagtagat	tgaggttcga	ggagttgtt	taggttgatt	agtgaaggat	780
aggttcgttag	ttattgttag	tttgggtttag	cgagtagagt	cgtttgcata	atatttagtag	840
gtgggttat	tttaaattaa	aaataagata	atttttaat	agttttgtt	tttagatatt	900
ttttttacgg	tttgggtttt	ttatttttgc	ttttttttaa	ttttttata	tttttttagt	960
ggtatttattt	ttgttttaga	aggcgcgaga	gtattatagg	gagtggggta	tatgggcgtg	1020
gggtgttattt	ttggggatag	acggagttgg	agttgtatag	gggataggtt	ggttgttaggg	1080
tgtattgtt	gggggtatag	tttttaagga	aaagagggtt	agtgtatagg	ttgtttttt	1140
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gaagtttttt	aggttagtt	gtcgtatttt	ttttttttt	atattgtta	taggattttt	1260
gttatttattt	attcgtgggt	tcgagggtt	tttttattac	ggtattttt	ttttttgtt	1320
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agaattttagt	tttaggggtt	tattatgtt	ataggttgc	agtaatattt	agttttttaa	1560
atatttatga	tagtaggtt	ggaggttgc	ttagtttgc	gttttacgtt	gtagaaagga	1620
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ggtaggtgag	gatataagtgt	ataattattt	gtgtataga	gtgtggatt	gggtgtaaat	1860
tgttttttag	gttaggggaa	agatattttt	gggagttttt	tggtgagttt	tggaaagggt	1920
tttagttttc	gaaggtttgg	ttttttattt	aggatatgtt	attttcgtcg	tttgagagta	1980
tttttattttt	atgttttaga	tttagtttaa	aattttttta	tttgcgtttt	gttaagta	2037

<210> 41

<211> 1542

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (*Homo sapiens*)

<400> 41

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ggttgcggga	agcgaaatat	cgtttttagc	gtttagggtt	tttttagaaat	atgagtataa	180
attgttttag	ttttttttt	cggcggtatc	ggtatacgta	tttagtattcg	tattgtatc	240
ggtatcggt	tttagtattcg	tattgtatc	ggtatacgta	tttagtattcg	tattgtatc	300
ggtatcggt	tttagtattcg	tattgtatc	ggtatacgta	tcgagcgtaa	gggttagggtc	360
gtcgaagtgc	gggtataatt	gtttagggtt	cgaattcggg	tttttagttt	gacgatattt	420
tttatagttt	gttcgaatgg	agcgttcgtt	ttgagtggcg	gttcgtttcg	gattcgttag	480
ttagttttta	gtggagtagc	tttttaattt	tgcgaggtcg	ttttttggagt	tttagtataat	540
attttttaat	tagtattatc	ggtttttagcg	agagtattga	tttttagttgt	taagagtgg	600
tttcgggggtt	ttagcgttta	taattcgagt	agtcggattt	ttaagtttat	tattagttcg	660
aatttttcg	atggggtcgt	tatagtttt	aattaggata	tcggatattt	ttgggtattta	720
gtaataggat	ttatttcggt	cgtaaaatttt	ttcgttaggt	tattgttaagg	gtttgttttt	780
tttttagggt	ttagtagttt	acggggttt	gtaaaaggat	cgattttgtt	ttcggatttt	840
aatttgattt	tagtggtcga	ttatatttgg	atatttgcac	ggggattttt	tatatttaat	900
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taaggggttgc	cgtatttta	gtgttaggtt	tttttgggtt	ttggtagtt	gattattttt	1020
atthaataat	agggttttag	tgtcgttata	agtatttttt	qtatttataa	tattttttag	1080

tgttaaggta ggggtttatt tttatTTtag tgTTTgatAT ttCGCgggGT ttaatataAG	1140
aATTTTTGt atTTAGTAAT TTTTTTAGT TGTcGATATA AGGATATTt AAATTTAATA	1200
ATTTcGTcG AGTGTtagTA TAAGGGTTcG TTTCGTTTT AGTGTtagT TTTTTcGGG	1260

tattagttga aatatttagtt tcgttttgg gcgtttcgg agtatttagta aaagggttcg	1320
tttcgtttat agtggtcgg tttttcgaa tattaaaaga aggatcggt tcgtttcgg	1380
gttttcggg ggagttgata gaagggttt ttttatttt tgcgtttt atttttgtgt	1440
ttacgattta ggagcgtgtt agttaaagta tggagaatta agagaaggcg agtacgcgg	1500
gttatatgtt cgacgtagtc gtgatcggag gtggatttt ag	1542

<210> 42
<211> 1542
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)

<400> 42

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taaagggtgg gaaggatTTT ttattaaatt ttttcggggaa gttcgggggc ggagtgcatt	180
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tttcgggagc gtttagggc ggagttgatg ttttagttga tattcggggg gagttggta	300
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tttgtgtcgg tagttggaaa ggattattgg gtgttaggagg ttttgtatt gaatttcgcg	420
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<210> 43
<211> 2866
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)

<400> 43

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attttcgtttt ttgggggtttt ttgggtcgg ttttttgcgtt ttgttacgag gttttgggtt ttattttttttt	180
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ttgagttgga cgttaagtag gtagaggta ttatggtaag aggtaggta ggtgttcggc	660
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gttggtagtt ttagtttag gtttatgtt aattttggg acgttagcgt ggatgtggag	780

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<210> 44

<211> 2866

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (*Homo sapiens*)

<400> 44

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<211> 1277

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 1277
<212> DNA
<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<400> 48

cracttacct tctactataa 20

<210> 49

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<212> DNA

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<223> AGT detection oligonucleotide

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18

<210> 50

<11> 18
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<400> 50

atatttttg gggttggg

18

专利名称(译)	诊断行为障碍，神经障碍和癌症		
公开(公告)号	EP1294948A2	公开(公告)日	2003-03-26
申请号	EP2001957909	申请日	2001-07-02
[标]申请(专利权)人(译)	埃皮吉諾米克斯股份公司		
申请(专利权)人(译)	AG EPIGENOMICS		
当前申请(专利权)人(译)	AG EPIGENOMICS		
[标]发明人	OLEK ALEXANDER PIEPENBROCK CHRISTIAN BERLIN KURT		
发明人	OLEK, ALEXANDER PIEPENBROCK, CHRISTIAN BERLIN, KURT		
IPC分类号	G01N27/62 C07K14/47 C07K14/82 C12M1/00 C12M1/34 C12N15/09 C12Q1/68 G01N33/53 G01N33/58 G01N37/00		
CPC分类号	C07K14/82 C07K14/4703 C12Q1/6883 C12Q1/6886 C12Q2523/125 C12Q2600/154 C12Q2600/156		
优先权	10032529 2000-06-30 DE 10043826 2000-09-01 DE		
外部链接	Espacenet		

摘要(译)

本发明涉及与行为障碍，神经障碍和癌症相关的基因的化学修饰的基因组序列，涉及用于检测与行为障碍，神经障碍和癌症相关的基因的胞嘧啶甲基化状态的寡核苷酸和/或PNA寡聚体。针对该序列，以及确定与行为障碍，神经障碍和癌症相关的基因的遗传和/或表观遗传参数的方法。