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(54) **DIAGNOSTIC MIRNA MARKERS FOR ALZHEIMER**

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G06F 19/22	(2011.01)

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CPC ***C12Q 1/6883*** (2013.01); ***C12Q 1/6809*** (2013.01); ***G06F 19/22*** (2013.01); *C12Q 2600/118* (2013.01); *C12Q 2600/158* (2013.01); *C12Q 2600/16* (2013.01); *C12Q 2600/178* (2013.01)

(58) **Field of Classification Search**

CPC C12Q 1/68
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,687,616 B1	3/2010	Bentwich et al.	
9,493,832 B2	11/2016	Vilanova et al.	
2009/0081640 A1	3/2009	Umansky et al.	
2010/0234445 A1	9/2010	Lui et al.	
2010/0280099 A1	11/2010	Elmen	
2012/0225925 A1*	9/2012	Sozzi	C12Q 1/6886 514/44 A
2015/0197805 A1	7/2015	Umansky et al.	

FOREIGN PATENT DOCUMENTS

CN	101861399 A	10/2010
EP	2077326 A1	7/2009
EP	2112235 A1	10/2009
WO	WO-2009/009457 A1	1/2009
WO	WO-2009/025852 A2	2/2009
WO	WO-2009/036236 A1	3/2009
WO	WO-2011/057003 A2	5/2011
WO	WO-2012/036433 A2	3/2012
WO	WO-2012/145363 A1	10/2012
WO	WO-2013/065819 A1	5/2013
WO	WO-2013/072920 A2	5/2013

OTHER PUBLICATIONS

Office Action dated Apr. 26, 2016 issued in U.S. Appl. No. 14/442,756.
NCBI Reference Sequence "*Homo sapiens* glutamate metabotropic receptor 7 (GRM7), RefSeqGene on chromosome 3".
Office Action dated Jun. 16, 2016 issued in U.S. Appl. No. 14/442,858.
Schulz, et al. (2002) Neuroscience Letters "Characterization of three novel isoforms of the metabotrobic glutamate receptor 7 (GRM7)".
Gao Kai et al., "Study on Parkinson's", Chinese Journal of Neurology (2011), 780-782.
Office Action dated Apr. 19, 2016, Issued in Chinese Patent Application No. 201380069445.9.
Regina Margis et al., "Identification of Blood microRNAs Associated to Parkinson's Disease", Journal of Biotechnology vol. 152 (2011) 96-101.
Search Report dated Apr. 19, 2016, issued in Chinese Patent Application No. 201380069445.9.
Kim et al. (2007), "A microRNA Feedback Circuit in Midbrain Dopamine Neurons", Science; 317(5842): pp. 1220-1224.
Martins et al. (2011), "Convergence of miRNA Expression Profiling, Alpha-Synuclein Interacton and GWAS in Parkinson's Disease", PLoS One, vol. 6, Iss 10; e25443; pp. 1-11.
Office Action dated Apr. 19, 2016 issued in co-pending Chinese Patent Application No. 2013/80069445.9.
Barbato, C., et al. (2009), "Searching for MIND: MicroRNAs in Neurodegenerative Diseases", *Journal of Biomedicine and Biotechnology*, 14(1): 8 pages.

(Continued)

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(57) **ABSTRACT**

The invention relates to methods for diagnosing Alzheimer's Disease (AD) with miRNA markers. Diagnosis of AD Towards the identification of biomarkers for diagnosis of AD, a comprehensive analysis of miRNA expression patterns was obtained. Significantly deregulated miRNAs were identified.

19 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

(56) **References Cited**

OTHER PUBLICATIONS

De Smaele, et al. (2010), "MicroRNAs as biomarkers for CNS cancer and other disorders", *Brain Res*. 1138: 100-111.
European Search Report dated Aug. 21, 2013 issued in European Patent Application No. 12192979.8.
European Search Report dated May 3, 2013 issued in European Patent Application No. 12192979.8.
International Search Report and Written Opinion dated Dec. 3, 2013 issued in PCT Patent Application No. PCT/EP2013/065819.
International Search Report and Written Opinion dated Mar. 7, 2014 issued in PCT Patent Application No. PCT/EP2013/072920.
Margis, R., et al. (2011), "Identification of blood microRNAs associated to Parkinsonis disease", *Journal of Biotechnology*, 152: 96-101.
Schipper, H., et al. (2007), "MicroRNA Expression in Alzheimer Blood Mononuclear Cells", *Gene Regulation and Systems Biology*, 1: 263-274.
Office Action dated Feb. 2, 2017 Issued in U.S. Appl. No. 14/442,858.
Office Action dated Apr. 10, 2017 issued in U.S. Appl. No. 14/442,756.
European Search Report dated Aug. 2, 2013 issued in European Patent Application No. 12192974.9.
FASTX—Toolkit; http://hannonlab.cshl.edu/fastx_toolkit/; retrieved onNov. 19, 2012, 10:16 a.m.
Geekiyanage, H., et al. (2012), "Blood serum miRNA: Non-invasive biomarkers for Alzheimer's disease", *Exp Neurol*. 235(2): 491-496.
Hudson, G., et al. (2012), "No consistent evidence for association between mtDNA variants and Alzheimer disease", *Neurology*, 78: 1038-1042.
International Search Report and Written Opinion dated Mar. 7, 2014 issued in PCT Patent Application No. PCT/EP2013/072567.
Keller, A., et al. (2011), "Toward the blood-borne miRNome of human diseases", *Nature Methods*, 8(10): 841-843.
Lange, J., et al. (2010), miRNA biomarkers from blood—A promising approach for minimally invasive diagnostic testing, *Gerburtshilfe Und Frauenheilkunde*, 70(2): 137-141—English Abstract Only.
Maes, O., et al. (2009), "MicroRNA: Implications for Alzheimer Disease and other Human CNS Disorders", *Current Genomics*, 10: 154-168.
Malaplate-Armand, C., et al. (2009), "Biomarkers for early diagnosis of Alzheimer's disease: Current update and future directions", *Revue Neurologique*, 165: 511-520—English Abstract Only.
Satoh, J. (2010), "MircoRNAs and Their Therapeutic Potential for Human Diseases: Aberrant MicroRNA Expression in Alzheimer's Disease Brains", *J. Pharmacol Sci*. 114: 269-275.
TAM tool; http://202.38.126.151/hmdd/tools/tam.html; retrieved on Nov. 19, 2012, 10:14 a.m.
Chinese Office Action dated May 27, 2016 in corresponding Chinese Patent Application CN 201380068818.0—English translation.
Friedländer, M., et al. (2012), "miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades", Nucleic Acids Research, 40(1): 37-52.
Office Action dated Aug. 29, 2016 issued in U.S. Appl. No. 14/442,756.
Office Action dated Oct. 23, 2017 issued in U.S. Appl. No. 14/442,756.
Biomarkers Definitions Working Group (2001) "Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework." Clinical Pharmacology & Therapeutics, v.69:89-95.
Office Action dated Apr. 26, 2018 issued in U.S. Appl. No. 14/442,858.
Strimbu, et al. (2010) "What are Biomarkers?" Curr. Opin. HIV AIDS V:5(6):463-6.
Office Action dated Oct. 9, 2018 issued in U.S. Appl. No. 14/442,858.

* cited by examiner

FIG 1

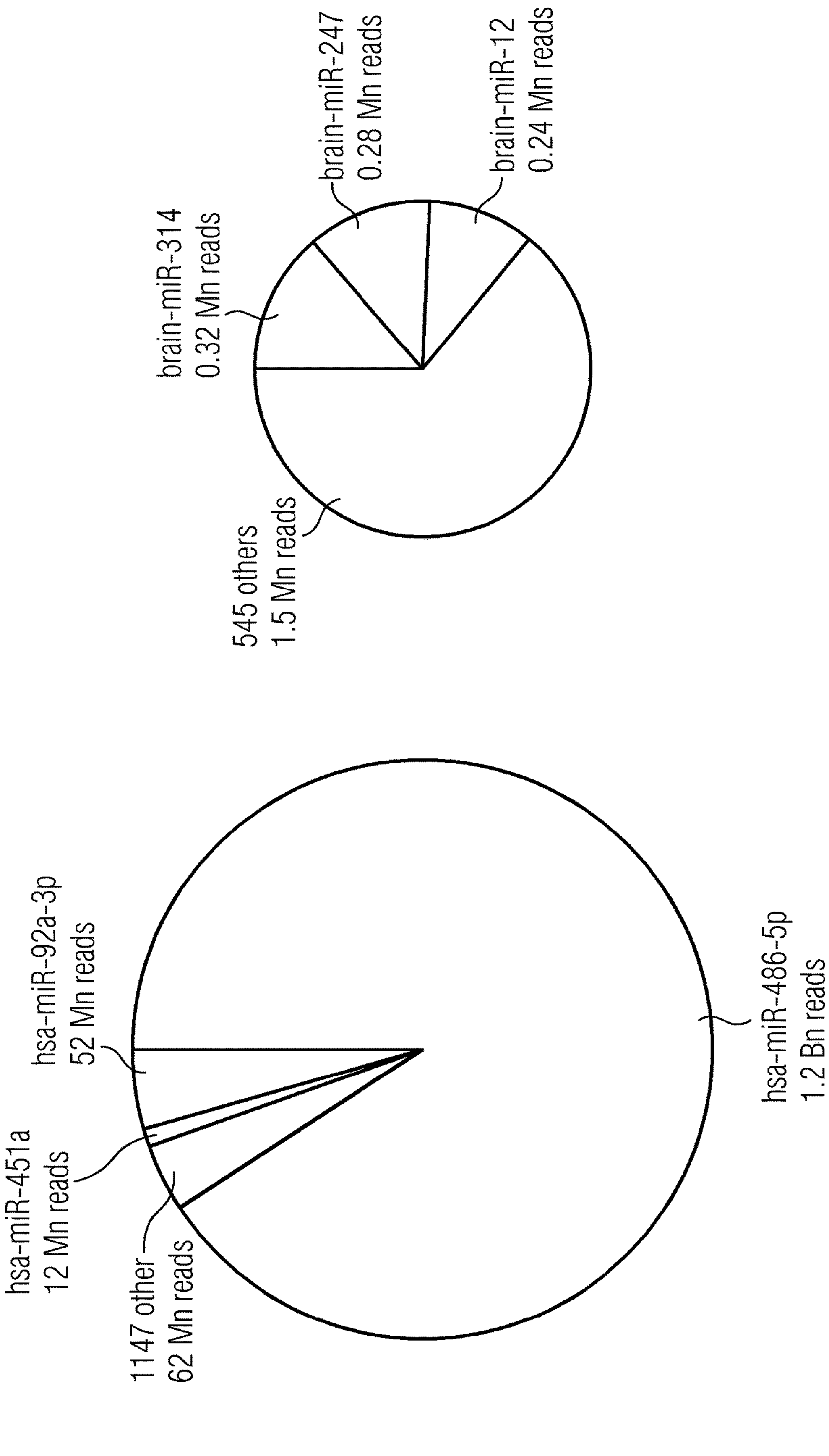

hsa-miR-92a-3p
52 Mn reads
hsa-miR-451a
12 Mn reads
1147 other
62 Mn reads
hsa-miR-486-5p
1.2 Bn reads
brain-miR-314
0.32 Mn reads
brain-miR-247
0.28 Mn reads
brain-miR-12
0.24 Mn reads
545 others
1.5 Mn reads

FIG 2

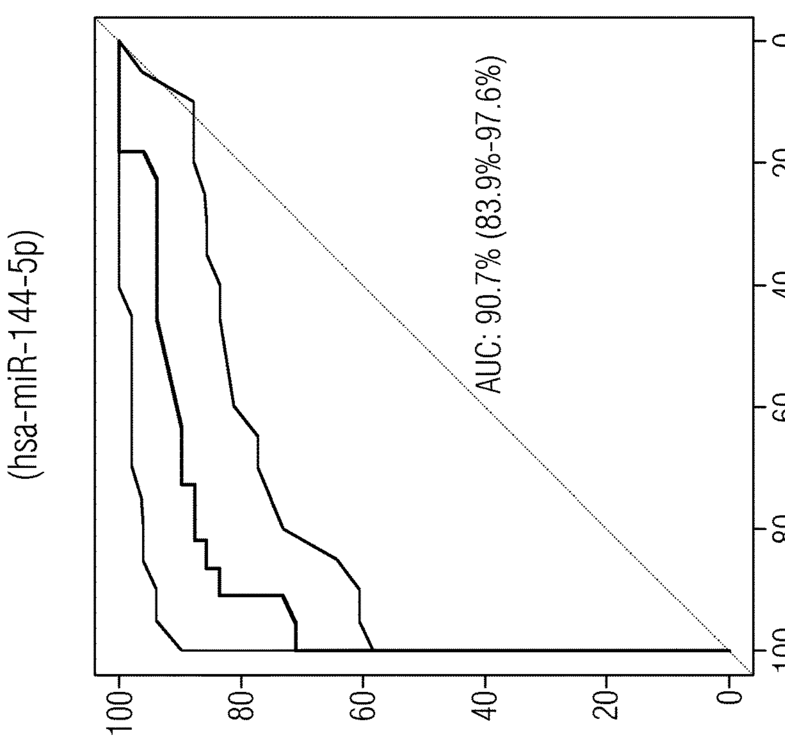

(hsa-miR-144-5p)
AUC: 90.7% (83.9%-97.6%)
100
80
60
40
20
0
0
20
40
60
80
100

FIG 3

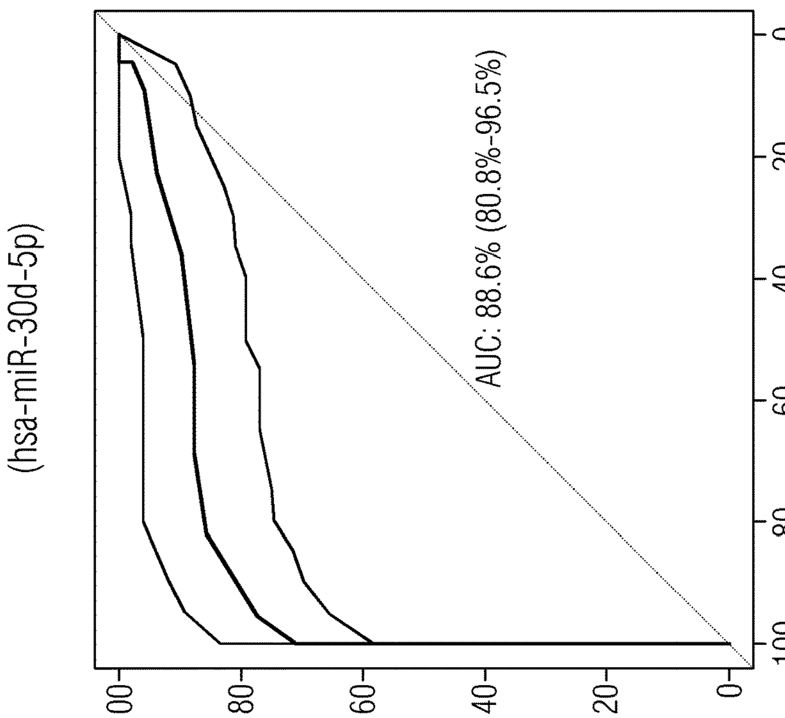

(hsa-miR-30d-5p)
AUC: 88.6% (80.8%-96.5%)
100
80
60
40
20
0
0
20
40
60
80
100

FIG 4

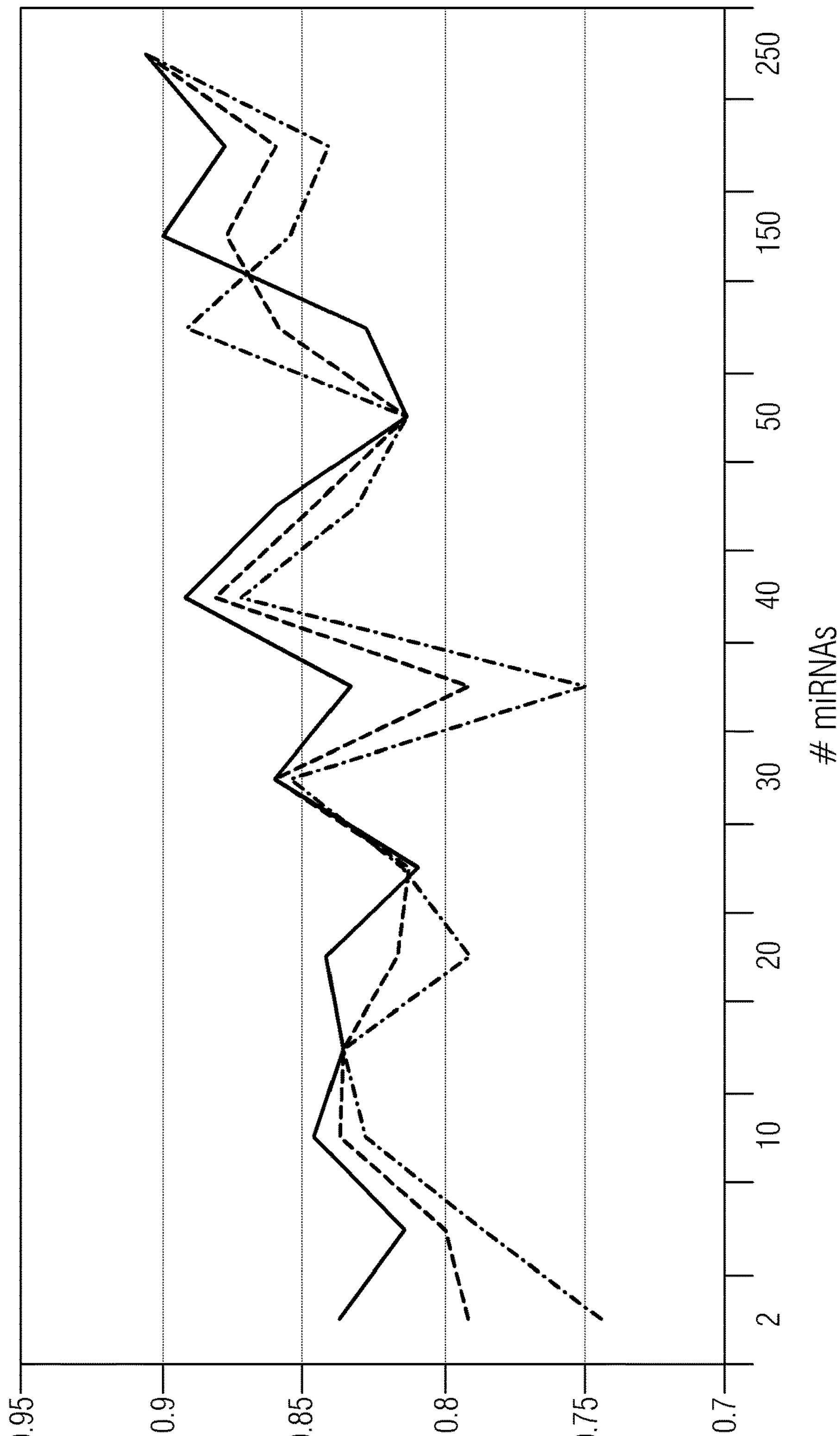


FIG 6

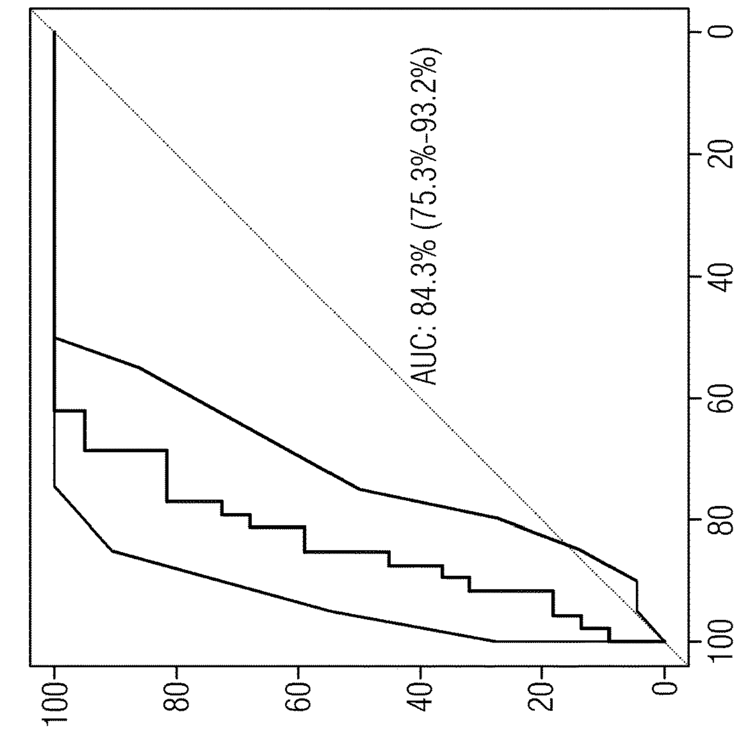

AUC: 84.3% (75.3%-93.2%)
100
80
60
40
20
0
0
20
40
60
80
100

FIG 5

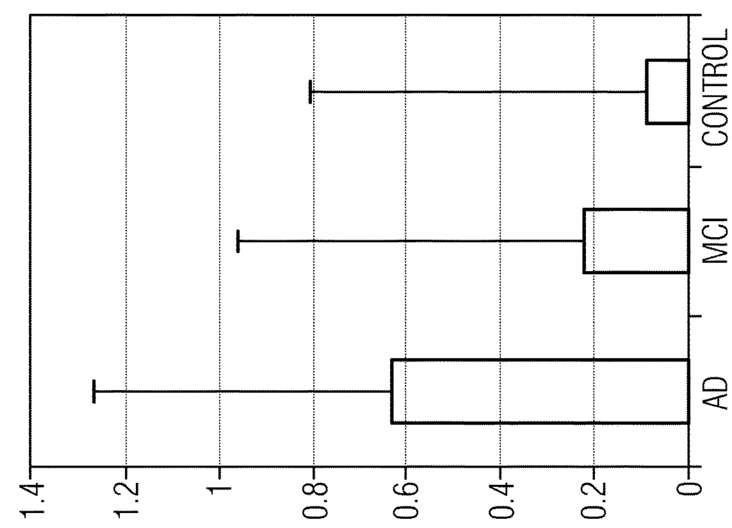
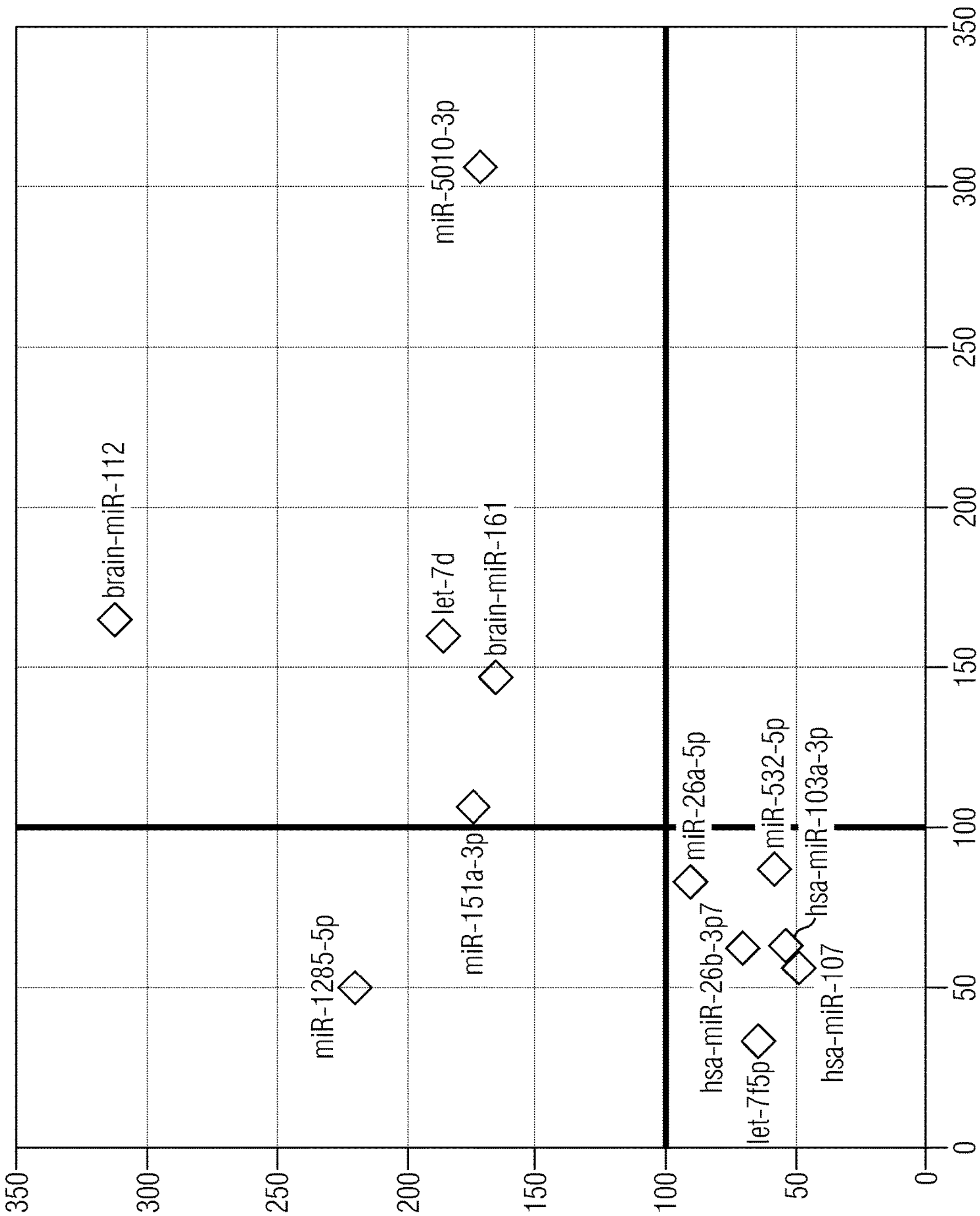

1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD
MCI
CONTROL

FIG 7



350
300
250
200
150
100
50
0
miR-5010-3p
brain-miR-112
let-7d
brain-miR-161
miR-151a-3p
miR-1285-5p
miR-26a-5p
miR-532-5p
hsa-miR-103a-3p
hsa-miR-26b-3p7
hsa-miR-107
let-7f5p

FIG 8

(hsa-miR-5010-3p)

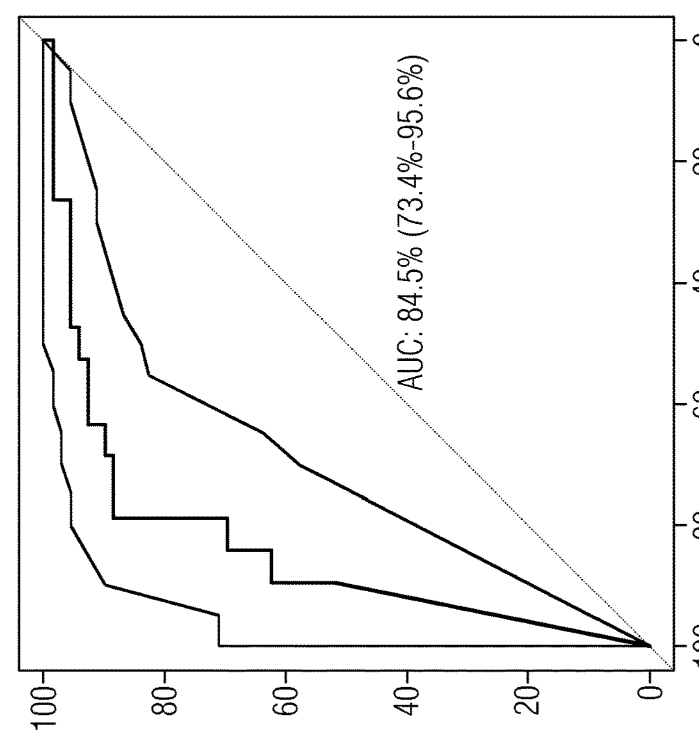
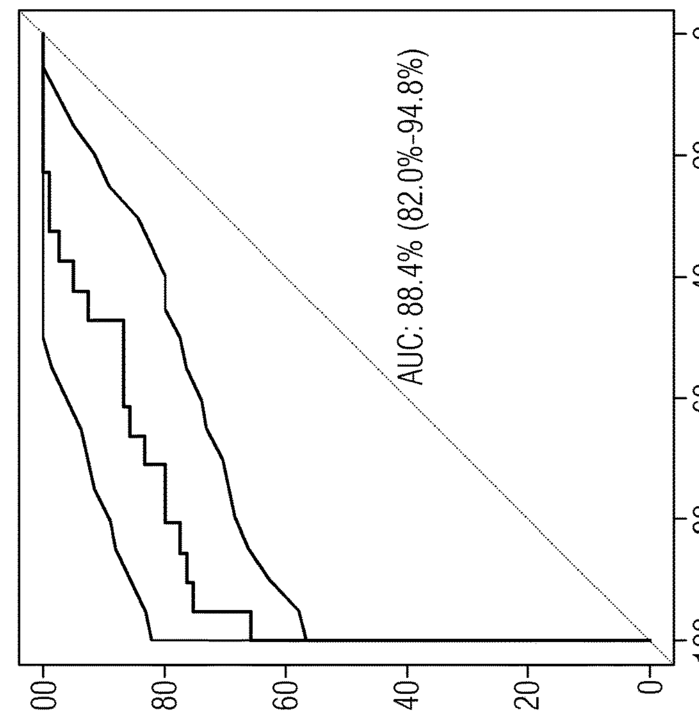


FIG 9



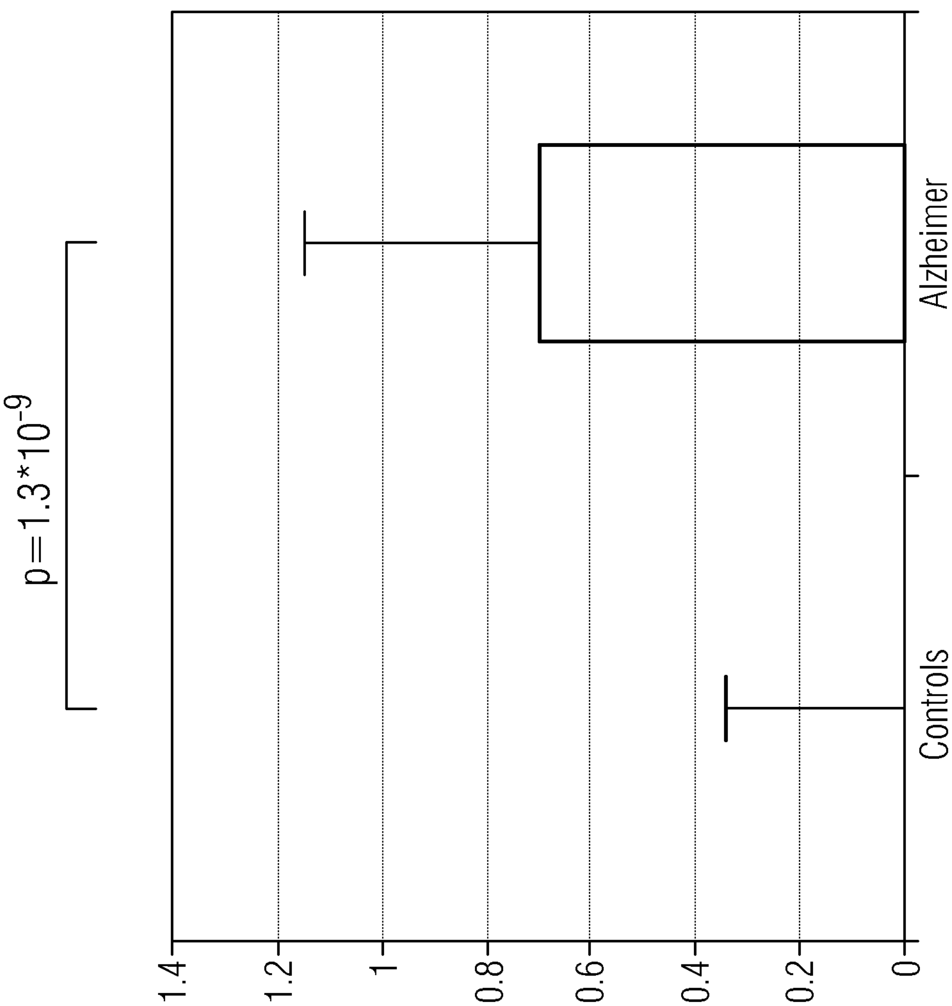


FIG 10

FIG 11

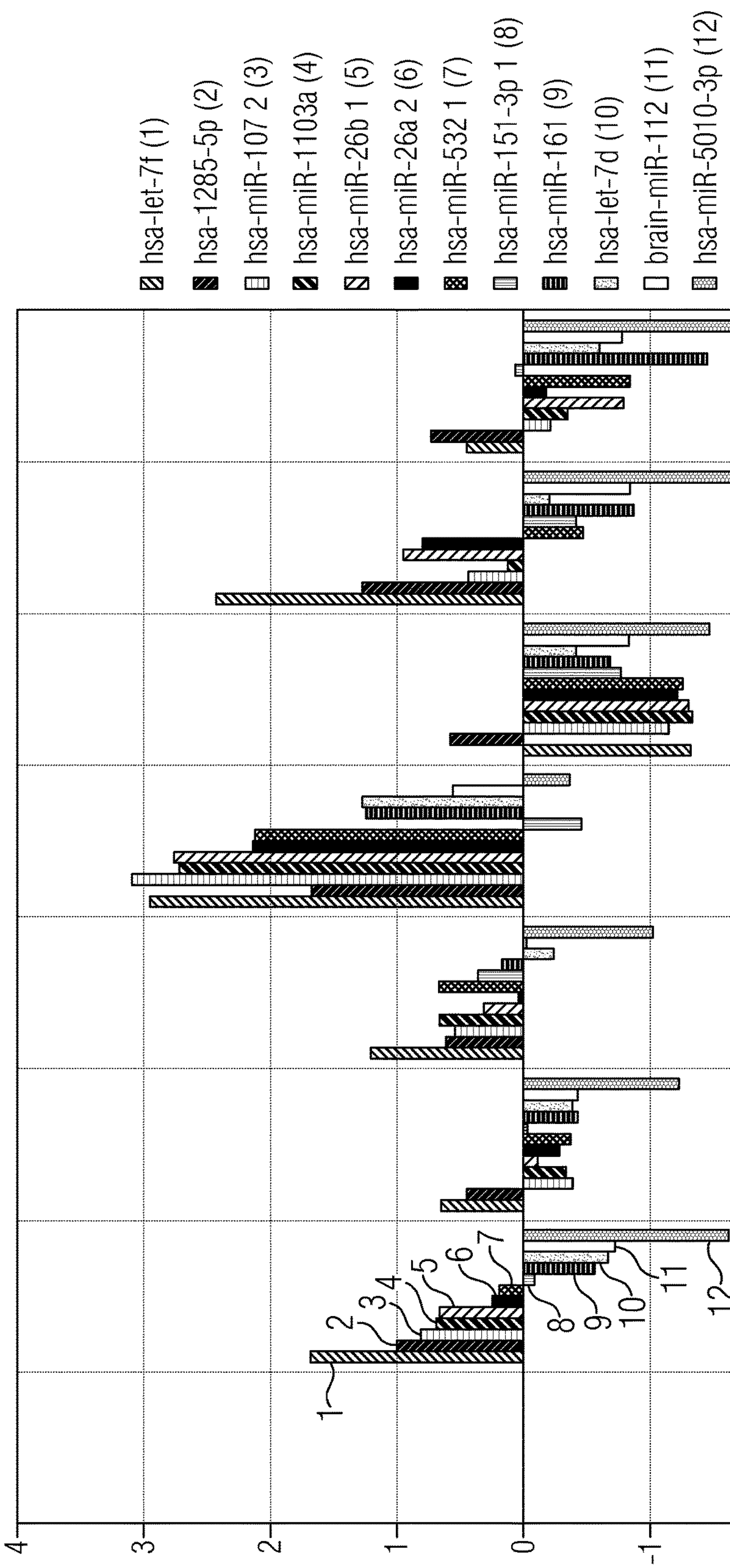

hsa-let-7f (1)
hsa-1285-5p (2)
hsa-miR-107 2 (3)
hsa-miR-1103a (4)
hsa-miR-26b 1 (5)
hsa-miR-26a 2 (6)
hsa-miR-532 1 (7)
hsa-miR-151-3p 1 (8)
hsa-miR-161 (9)
hsa-let-7d (10)
brain-miR-112 (11)
hsa-miR-5010-3p (12)
4
3
2
1
0
-1
-2
-3
Control
AD
MCI
PD
DEP
CIS
SCH
BD
1
2
3
4
5
6
7
8
9
10
11
12

FIG 12

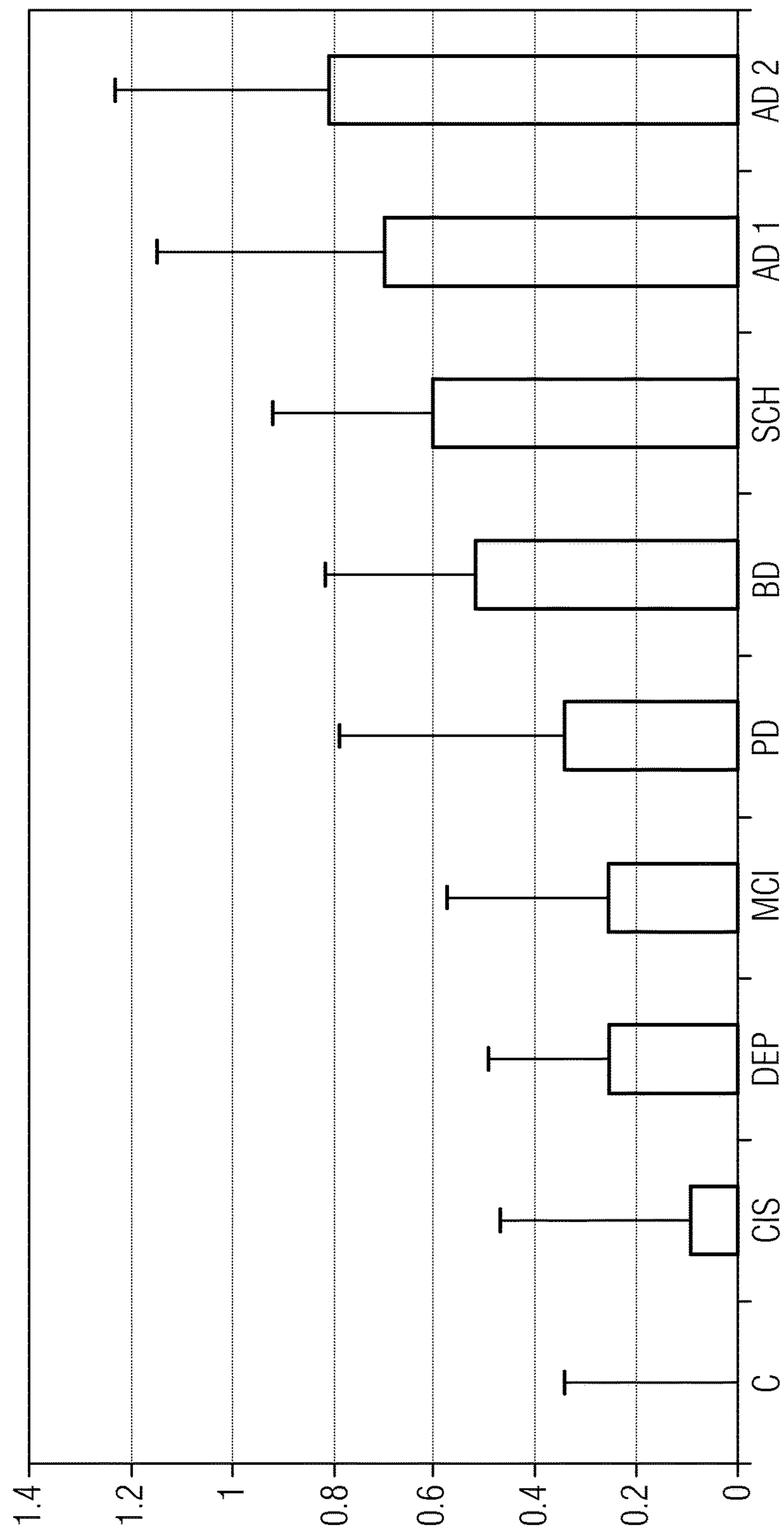


FIG 13

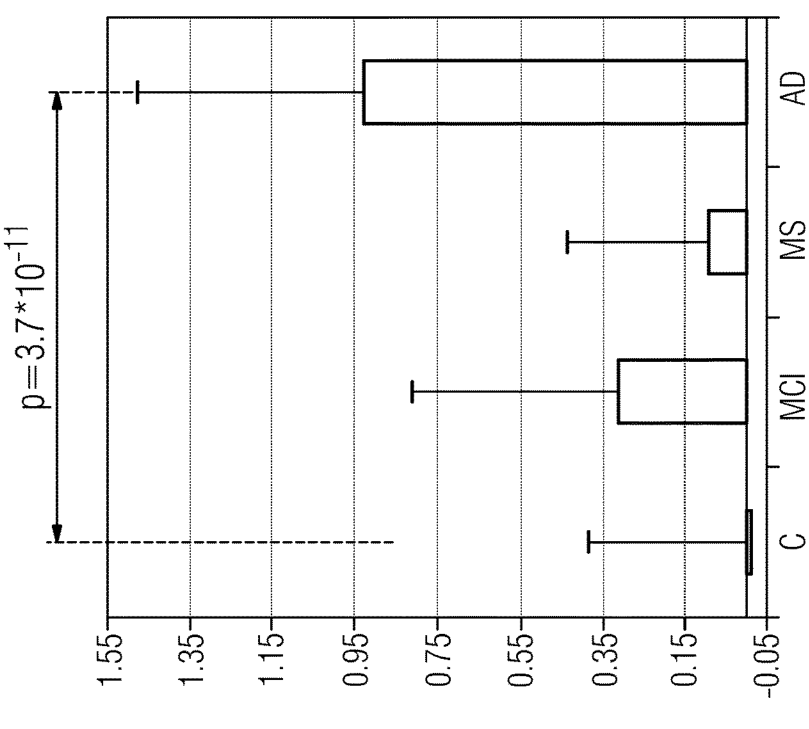

p=3.7*10^-11
1.55
1.35
1.15
0.95
0.75
0.55
0.35
0.15
-0.05
C
MCI
MS
AD

FIG 14

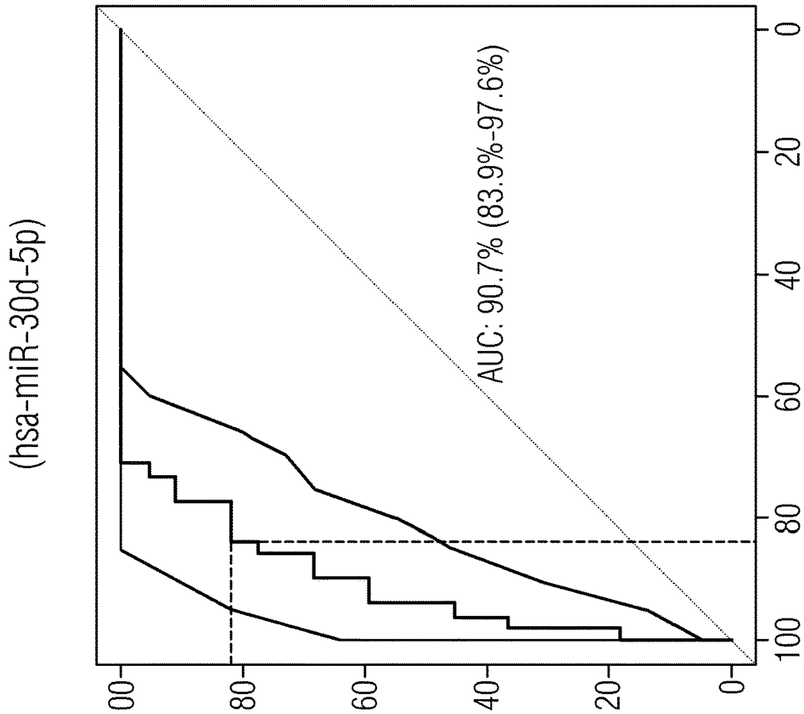

(hsa-miR-30d-5p)
AUC: 90.7% (83.9%-97.6%)
100
80
60
40
20
0

FIG 15

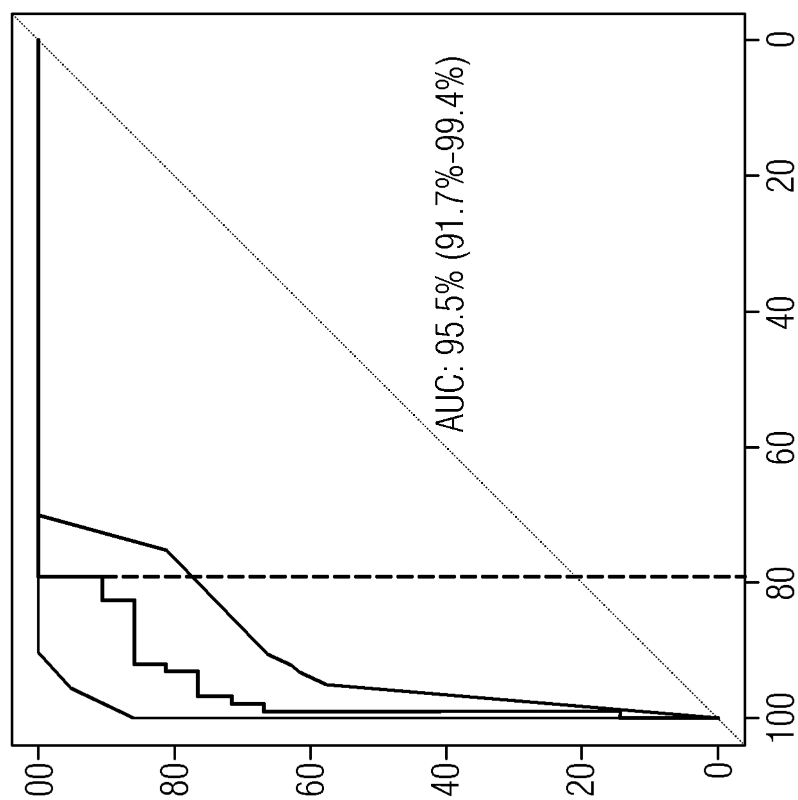
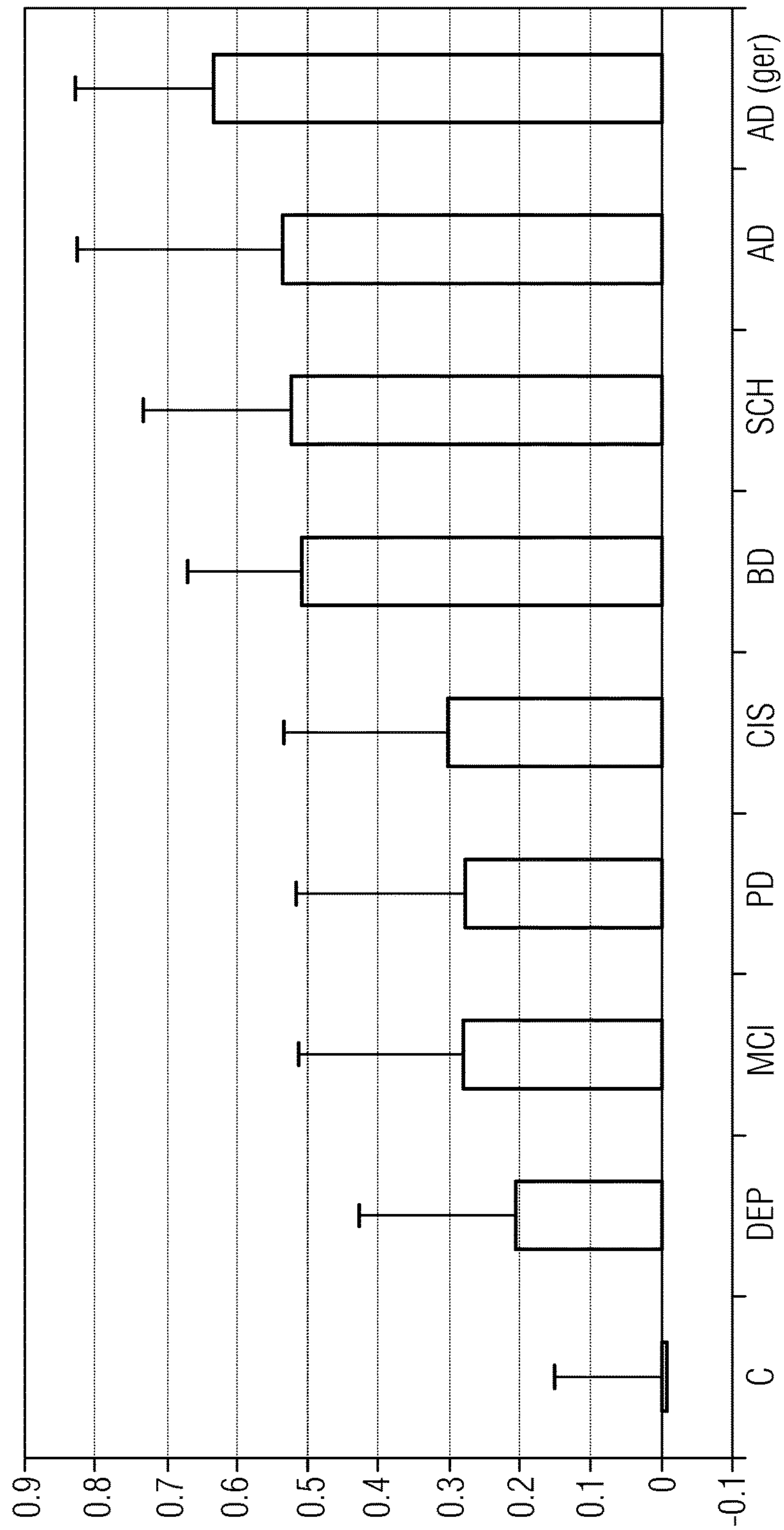


FIG 16



DIAGNOSTIC MIRNA MARKERS FOR ALZHEIMER

PRIORITY STATEMENT

This application is a national phase application under 35 U.S.C. § 371 of PCT International Application No. PCT/EP2013/072567 which has an International filing date of 29 Oct. 2013, which designated the United States of America, and which claims priority to European patent application number 12192974.9 filed 16 Nov. 2012. The entire contents of each patent application referenced above are hereby incorporated by reference.

REFERENCE TO A SEQUENCE LISTING

This application contains references to amino acid sequences and/or nucleic acid sequences which have been submitted concurrently herewith as the sequence listing text file 62095355_1.TXT file size 34 KiloBytes (KB), created on 1 Nov. 2017. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. § 1.52(e)(5).

FIELD OF THE INVENTION

The invention relates to novel markers for diagnosing Alzheimer's disease.

BACKGROUND OF THE INVENTION

Very recently, molecular diagnostics has increasingly gained in importance. It has found an entry into the clinical diagnosis of diseases (inter alia detection of infectious pathogens, detection of mutations of the genome, detection of diseased cells and identification of risk factors for predisposition to a disease).

In particular, through the determination of gene expression in tissues, nucleic acid analysis opens up very promising new possibilities in the study and diagnosis of disease.

Nucleic acids of interest to be detected include genomic DNA, expressed mRNA and other RNAs such as MicroRNAs (abbreviated miRNAs). MiRNAs are a new class of small RNAs with various biological functions (A. Keller et al., Nat Methods. 2011 8(10):841-3). They are short (average of 20-24 nucleotide) ribonucleic acid (RNA) molecules found in eukaryotic cells. Several hundred different species of microRNAs (i.e. several hundred different sequences) have been identified in mammals. They are important for post-transcriptional gene-regulation and bind to complementary sequences on target messenger RNA transcripts (mRNAs), which can lead to translational repression or target degradation and gene silencing. As such they can also be used as biologic markers for research, diagnosis and therapy purposes.

Alzheimer's disease (AD), also known in medical literature as Alzheimer disease, is the most common form of dementia. Alzheimer's disease is characterised by loss of neurons and synapses in the cerebral cortex and certain subcortical regions and leads to a gross degeneration in these regions. In AD protein misfolding and aggregation (formation of so-called "plaques") in the brain is caused by accumulation of abnormally folded A-beta and tau proteins in the affected tissues.

Early symptoms are often mistaken to be age-related problems. In the early stages, the most common symptom is difficulty in remembering recent events. When AD is suspected, the diagnosis is usually confirmed with functional tests that evaluate behaviour and cognitive abilities, often followed by imaging analysis of the brain. Imaging methods used for this purpose include computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), and positron emission tomography (PET). In a patients already having dementia, SPECT appears to be superior in differentiating Alzheimer's disease from other possible causes, compared with the usual attempts employing mental testing and medical history analysis. A new technique known as PiB PET has been developed for directly and clearly imaging beta-amyloid deposits in vivo using a tracer that binds selectively to the beta-amyloid deposits. Beta-amyloid deposits. Recently, a miRNA diagnostic test from serum has been proposed (Geekiyanage et al., Exp Neurol. 2012 June; 235(2):491-6.)

Symptoms can be similar to other neurological disorders. Diagnosis can be time consuming, expensive and difficult. In particular, the reliable and early diagnosis of Alzheimer based on non-invasive molecular biomarkers remains a challenge. Till today, early diagnosis of AD remains a great challenge. So far, findings of an autopsy or biopsy represent the most reliable diagnostics for this common disease

The attempt to report the presence of beta-amyloid not only in the brain, but also in other tissues, e.g. the skin, showed only limited relevance for diagnosing AD. (Malaplate-Armand C, Desbene C, Pillot T, Olivier J L. Diagnostic biologique de la maladie d'Alzheimer: avancées, limites et perspectives. Rev Neurol 2009; 165:511-520). Thus, in the recent past, different imaging as well as in vitro diagnostic markers have been proposed in order to improve the AD diagnosis. Most importantly, biomarkers that can detect AD in pre-clinical stages are in the focus, however, such markers can so far be only reliably detected in cerebrospinal fluid (CSF). One prominent example is the combination of beta-amyloid-1-42 and tau. In addition, molecular genetics analyses of single nucleotide polymorphisms (SNPs) in the DNA of patients have been proposed to provide a risk estimation of the presence of AD. In addition to variants in genes, several studies have described an association between AD and genetic variation of mitochondrial DNA (mtDNA). Here, no consistent evidence for the relation of mtDNA variants and AD could be reported Hudson G, Sims R, Harold D, et al.; GERAD1 Consortium. No consistent evidence for association between mtDNA variants and Alzheimer disease. Neurology 2012; 78:1038-1042. However, although the heritability of AD is comparably high (60-80%), epigenetic and persistent factors also may play an important role.

Therefore, there exists an unmet need for an efficient, simple, reliable diagnostic test for AD.

OBJECT OF THE INVENTION

The technical problem underlying the present invention is to provide biological markers allowing to diagnose, screen for or monitor Alzheimer's disease, predict the risk of developing Alzheimer's disease, or predict an outcome of Alzheimer's disease.

SUMMARY OF THE INVENTION

Before the invention is described in detail, it is to be understood that this invention is not limited to the particular component parts of the process steps of the methods described as such methods may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include singular and/or plural referents unless the context clearly dictates otherwise. It is also to be understood that plural forms include singular and/or plural referents unless the context clearly dictates otherwise. It is moreover to be understood that, in case parameter ranges are given which are delimited by numeric values, the ranges are deemed to include these limitation values.

In its most general terms, the invention relates to a collection of miRNA markers useful for the diagnosis, prognosis and prediction of Alzheimer’s Disease.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows the distribution of reads obtained by high throughput sequencing. The left pie chart shows that 95% of known miRNAs belong to one miRNA while all other 1.000 detected known markers make up only 5%. The novel detected miRNAs on the right hand side are much less abundant than the most frequently detected miRNA.

FIG. **2** shows the ROC curve for the most up-regulated miRNA, gene expression data obtained by NGS. X-axis: specificity, y-axis: sensitivity.

FIG. **3** shows the ROC curve for the most down-regulated miRNA, gene expression data obtained by NGS. X-axis: specificity, y-axis: sensitivity.

FIG. **4** shows increased performance by using marker combinations. The x-axis shows the number of miRNAs, the y axis shows the classification of performance, gene expression data obtained by NGS (solid line: sensitivity, broken line: accuracy, broken and dotted line: specificity).

FIG. **5** shows a combined score of AD, MCI and controls for the 7-marker signature brain-mir-112, hsa-miR-5010-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-let-7d-3p, hsa-miR-532-5p, and brain-mir-161. The combined score (y-axis) was obtained using high throughput sequencing.

FIG. **6** shows the ROC curve for the 7-marker signature of FIG. **5**, gene expression data obtained by NGS.

FIG. **7** shows the qRT-PCR validation of selected miRNAs, the up-regulated miRNAs brain-mir-112, brain-mir-161, hsa-let-7d-3p, hsa-miR-5010-3p, hsa-miR-26b-3p, hsa-miR-26a-5p, hsa-miR-1285-5p, and hsa-miR-151a-3p as well as the down-regulated markers hsa-miR-103a-3p, hsa-miR-107, hsa-miR-532-5p, and hsa-let-7f-5p. X-axis: expression of AD samples vs. control determined by NGS, y-axis: expression of AD samples vs. control determined by qRT-PCR.

FIG. **8** shows the ROC curve for the best single miRNAs from the validation study, gene expression data obtained by qRT-PCR. X-axis: specificity, y-axis: sensitivity.

FIG. **9** shows the ROC curve for the 7-marker signature brain-mir-112, hsa-miR-5010-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-let-7d-3p, hsa-miR-532-5p, and brain-mir-161, qRT-PCR. X-axis: specificity, y-axis: sensitivity.

FIG. **10** shows the improved combined score of controls (left column) vs. AD patients (right column).

FIG. **11** shows the validation of 12 miRNAs in 7 diseases (AD, MCI, PD, DEP, CIS, SCH, and BD and controls). The 12 miRNAs are (denoted by columns 1-12, respectively) hsa-let-7f-5p, hsa-miR-1285-5p, hsa-miR-107, hsa-miR-103a-3p, hsa-miR-26b-3p, hsa-miR-26a-5p, hsa-miR-532-5p, hsa-miR-151a-3p, brain-mir-161, hsa-let-7d-3p, brain-mir-112, and hsa-miR-5010-3p.

FIG. **12** shows the combined score of the 7-miRNA signature brain-mir-112, hsa-miR-5010-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-let-7d-3p, hsa-miR-532-5p, and brain-mir-161 for all diseases. The combined score (y-axis) was obtained using quantitative RT PCR.

FIG. **13** shows a combined score of AD, MCI and controls for the 12-marker signature hsa-let-7f-5p, hsa-miR-1285-5p, hsa-miR-107, hsa-miR-103a-3p, hsa-miR-26b-3p, hsa-miR-26a-5p, hsa-miR-532-5p, hsa-miR-151a-3p, brain-mir-161, hsa-let-7d-3p, brain-mir-112, and hsa-miR-5010-3p. The combined score (y-axis) was obtained using high throughput sequencing.

FIG. **14** shows the ROC curve for the 12-marker signature of FIG. **13**, gene expression data obtained by NGS. X-axis: specificity, y-axis: sensitivity.

FIG. **15** shows the ROC curve for the 12-marker signature of FIG. **13**, gene expression data obtained by qRT-PCR. X-axis: specificity, y-axis: sensitivity.

FIG. **16** shows the combined score of the 12-miRNA signature hsa-let-7f-5p, hsa-miR-1285-5p, hsa-miR-107, hsa-miR-103a-3p, hsa-miR-26b-3p, hsa-miR-26a-5p, hsa-miR-532-5p, hsa-miR-151a-3p, brain-mir-161, hsa-let-7d-3p, brain-mir-112, and hsa-miR-5010-3p for all diseases. The combined score (y-axis) was obtained using quantitative RT PCR.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term “predicting an outcome” of a disease, as used herein, is meant to include both a prediction of an outcome of a patient undergoing a given therapy and a prognosis of a patient who is not treated.

An “outcome” within the meaning of the present invention is a defined condition attained in the course of the disease. This disease outcome may e.g. be a clinical condition such as “relapse of disease”, “remission of disease”, “response to therapy”, a disease stage or grade or the like.

A “risk” is understood to be a probability of a subject or a patient to develop or arrive at a certain disease outcome. The term “risk” in the context of the present invention is not meant to carry any positive or negative connotation with regard to a patient’s wellbeing but merely refers to a probability or likelihood of an occurrence or development of a given event or condition.

The term “clinical data” relates to the entirety of available data and information concerning the health status of a patient including, but not limited to, age, sex, weight, menopausal/hormonal status, etiopathology data, anamnesis data, data obtained by in vitro diagnostic methods such as blood or urine tests, data obtained by imaging methods, such as x-ray, computed tomography, MRI, PET, spect, ultrasound, electrophysiological data, genetic analysis, gene expression analysis, biopsy evaluation, intraoperative findings.

The term “classification of a sample” of a patient, as used herein, relates to the association of said sample with at least one of at least two categories. These categories may be for example “high risk” and “low risk”; or high, intermediate and low risk; wherein risk is the probability of a certain event occurring in a certain time period, e.g. occurrence of disease, progression of disease, etc. It can further mean a category of favourable or unfavourable clinical outcome of disease, responsiveness or non-responsiveness to a given treatment or the like. Classification may be performed by use of an algorithm, in particular a discriminate function. A simple example of an algorithm is classification according to a first quantitative parameter, e.g. expression level of a nucleic acid of interest, being above or below a certain threshold value. Classification of a sample of a patient may be used to predict an outcome of disease or the risk of developing a disease. Instead of using the expression level of a single nucleic acid of interest, a combined score of several nucleic acids of interest of interest may be used. Further, additional data may be used in combination with the first quantitative parameter. Such additional data may be clinical data from the patient, such as sex, age, weight of the patient, disease grading etc.

A "discriminant function" is a function of a set of variables used to classify an object or event. A discriminant function thus allows classification of a patient, sample or event into a category or a plurality of categories according to data or parameters available from said patient, sample or event. Such classification is a standard instrument of statistical analysis well known to the skilled person. E.g. a patient may be classified as "high risk" or "low risk", "in need of treatment" or "not in need of treatment" or other categories according to data obtained from said patient, sample or event. Classification is not limited to "high vs. low", but may be performed into a plurality of categories, grading or the like. Examples for discriminant functions which allow a classification include, but are not limited to discriminant functions defined by support vector machines (SVM), k-nearest neighbors (kNN), (naive) Bayes models, or piecewise defined functions such as, for example, in subgroup discovery, in decision trees, in logical analysis of data (LAD) an the like.

The term "expression level" refers, e.g., to a determined level of expression of a nucleic acid of interest. The term "pattern of expression levels" refers to a determined level of expression com-pared either to a reference nucleic acid, e.g. from a control, or to a computed average expression value, e.g. in DNA-chip analyses. A pattern is not limited to the comparison of two genes but is also related to multiple comparisons of genes to reference genes or samples. A certain "pattern of expression levels" may also result and be determined by comparison and measurement of several nucleic acids of interest disclosed hereafter and display the relative abundance of these transcripts to each other. Expression levels may also be assessed relative to expression in different tissues, patients versus healthy controls, etc.

A "reference pattern of expression levels", within the meaning of the invention shall be understood as being any pattern of expression levels that can be used for the comparison to another pattern of expression levels. In a preferred embodiment of the invention, a reference pattern of expression levels is, e.g., an average pattern of expression levels observed in a group of healthy or diseased individuals, serving as a reference group.

In the context of the present invention a "sample" or a "biological sample" is a sample which is derived from or has been in contact with a biological organism. Examples for biological samples are: cells, tissue, body fluids, biopsy specimens, blood, urine, saliva, sputum, plasma, serum, cell culture supernatant, and others.

A "probe" is a molecule or substance capable of specifically binding or interacting with a specific biological molecule. The term "primer", "primer pair" or "probe", shall have ordinary meaning of these terms which is known to the person skilled in the art of molecular biology. In a preferred embodiment of the invention "primer", "primer pair" and "probes" refer to oligonucleotide or polynucleotide molecules with a sequence identical to, complementary too, homologues of, or homologous to regions of the target molecule or target sequence which is to be detected or quantified, such that the primer, primer pair or probe can specifically bind to the target molecule, e.g. target nucleic acid, RNA, DNA, cDNA, gene, transcript, peptide, polypeptide, or protein to be detected or quantified. As understood herein, a primer may in itself function as a probe. A "probe" as understood herein may also comprise e.g. a combination of primer pair and internal labeled probe, as is common in many commercially available qPCR methods.

A "gene" is a set of segments of nucleic acid that contains the information necessary to produce a functional RNA product in a controlled manner. A "gene product" is a biological molecule produced through transcription or expression of a gene, e.g. an mRNA or the translated protein.

A "miRNA" is a short, naturally occurring RNA molecule and shall have the ordinary meaning understood by a person skilled in the art. A "molecule derived from an miRNA" is a molecule which is chemically or enzymatically obtained from an miRNA template, such as cDNA.

The term "array" refers to an arrangement of addressable locations on a device, e.g. a chip device. The number of locations can range from several to at least hundreds or thousands. Each location represents an independent reaction site. Arrays include, but are not limited to nucleic acid arrays, protein arrays and antibody-arrays. A "nucleic acid array" refers to an array containing nucleic acid probes, such as oligonucleotides, polynucleotides or larger portions of genes. The nucleic acid on the array is preferably single stranded. A "microarray" refers to a biochip or biological chip, i.e. an array of regions having a density of discrete regions with immobilized probes of at least about 100/cm2.

A "PCR-based method" refers to methods comprising a polymerase chain reaction PCR. This is a method of exponentially amplifying nucleic acids, e.g. DNA or RNA by enzymatic replication in vitro using one, two or more primers. For RNA amplification, a reverse transcription may be used as a first step. PCR-based methods comprise kinetic or quantitative PCR (qPCR) which is particularly suited for the analysis of expression levels). When it comes to the determination of expression levels, a PCR based method may for example be used to detect the presence of a given mRNA by (1) reverse transcription of the complete mRNA pool (the so called transcriptome) into cDNA with help of a reverse transcriptase enzyme, and (2) detecting the presence of a given cDNA with help of respective primers. This approach is commonly known as reverse transcriptase PCR (rtPCR). The term "PCR based method" comprises both end-point PCR applications as well as kinetic/real time PCR techniques applying special fluorophors or intercalating dyes which emit fluorescent signals as a function of amplified target and allow monitoring and quantification of the target. Quantification methods could be either absolute by external standard curves or relative to a comparative internal standard.

The term "next generation sequencing" or "high throughput sequencing" refers to high-throughput sequencing technologies that parallelize the sequencing process, producing thousands or millions of sequences at once. Examples include Massively Parallel Signature Sequencing (MPSS) Polony sequencing, 454 pyrosequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Ion semiconductor sequencing, DNA nanoball sequencing, Helioscope™ single molecule sequencing, Single Molecule SMR™ sequencing, Single Molecule real time (RNAP) sequencing, Nanopore DNA sequencing.

The term "marker" or "biomarker" refers to a biological molecule, e.g., a nucleic acid, peptide, protein, hormone, etc., whose presence or concentration can be detected and correlated with a known condition, such as a disease state, or with a clinical outcome, such as response to a treatment.

In particular, the invention relates to a method of classifying a sample of a patient suffering from or at risk of developing Alzheimer's Disease, wherein said sample is a blood sample, said method comprising the steps of:

a) determining in said sample an expression level of at least one miRNA selected from the group consisting of miRNAs having the sequence SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1 to SEQ ID NO 58, SEQ ID NO 60 to SEQ ID NO 64 and SEQ ID NO 66 to SEQ ID NO 170,

b) comparing the pattern of expression level(s) determined in step a) with one or several reference pattern(s) of expression levels; and

c) classifying the sample of said patient from the outcome of the comparison in step b) into one of at least two classes.

A reference pattern of expression levels may, for example, be obtained by determining in at least one healthy subject the expression level of at least one miRNA selected from the group consisting of miRNAs having the sequence SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1 to SEQ ID NO 58, SEQ ID NO 60 to SEQ ID NO 64 and SEQ ID NO 66 to SEQ ID NO 170.

It is within the scope of the invention to assign a numerical value to an expression level of the at least one miRNA determined in step a).

It is further within the scope of the invention to mathematically combine expression level values to obtain a pattern of expression levels in step (b), e.g. by applying an algorithm to obtain a normalized expression level relative to a reference pattern of expression level(s).

In a further aspect the invention relates to a method for diagnosing Alzheimer's Disease, predicting risk of developing Alzheimer's Disease, or predicting an outcome of Alzheimer's Disease in a patient suffering from or at risk of developing Alzheimer's Disease, said method comprising the steps of:

a) determining in a blood sample from said patient, the expression level of at least one miRNA selected from the group consisting of miRNAs with the sequence SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1 to SEQ ID NO 58, SEQ ID NO 60 to SEQ ID NO 64 and SEQ ID NO 66 to SEQ ID NO 170,

b) comparing the pattern of expression level(s) determined in step a) with one or several reference pattern(s) of expression levels; and

c) diagnosing Alzheimer's Disease, predicting a risk of developing Alzheimer's Disease, or predicting an outcome of Alzheimer's Disease from the outcome of the comparison in step b).

According to an aspect of the invention, said at least one miRNA is selected from the group consisting of miRNAs with the sequence SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1 and SEQ ID NO 56.

According to an aspect of the invention, step a) comprises determining the expression level of the miRNAs: brain-mir-112, hsa-miR-5010-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-let-7d-3p, hsa-miR-532-5p, and brain-mir-161.

According to an aspect of the invention, step a) comprises in step a) determining the expression level of 5 miRNAs selected from the signatures consisting of

brain-mir-112 hsa-miR-5010-3p hsa-miR-1285-5p hsa-miR-151a-3p hsa-let-7f-5p,
hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-3157-3p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-1285-5p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7a-5p,
hsa-miR-5001-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,
hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-miR-144-5p hsa-let-7a-5p,
hsa-miR-1285-5p brain-mir-112 hsa-miR-425-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-5001-3p hsa-miR-1285-5p brain-mir-112 hsa-miR-151a-3p hsa-let-7f-5p,
hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-148b-5p hsa-miR-144-5p hsa-let-7f-5p,
hsa-miR-1285-5p hsa-miR-3157-3p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-5001-3p hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p,
brain-mir-431 hsa-miR-1285-5p hsa-miR-3157-3p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-1285-5p brain-mir-112 hsa-miR-425-5p hsa-miR-151a-3p,
hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-miR-144-5p hsa-let-7f-5p,
hsa-miR-550a-5p hsa-miR-1285-5p brain-mir-112 hsa-miR-151a-3p hsa-let-7f-5p,
hsa-miR-1285-5p brain-mir-112 hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p, and
hsa-miR-5001-3p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p.

According to an aspect of the invention, the expression levels of a plurality of miRNAs are determined as expression level values and step (b) comprises mathematically combining the expression level values of said plurality of miRNAs.

It is within the scope of the invention to apply an algorithm to the numerical value of the expression level of the at least one miRNA determined in step a) to obtain a disease score to allow classification of the sample or diagnosis, prognosis or prediction of the risk of developing Alzheimer's Disease, or prediction of an outcome of Alzheimer's Disease. A non-limiting example of such an algorithm is to compare the numerical value of the expression level against a threshold value in order to classify the result into one of two categories, such as high risk/low risk, diseased/healthy or the like. A further non-limiting example of such an algorithm is to combine a plurality of numerical values of expression levels, e.g. by summation, to obtain a combined score. Individual summands may be normalized or weighted by multiplication with factors or numerical values representing the expression level of an miRNA, numerical values representing clinical data, or other factors.

It is within the scope of the invention to apply a discriminant function to classify a result, diagnose disease, predict an outcome or a risk.

According to an aspect of the invention, the expression level in step (a) is obtained by use of a method selected from the group consisting of a Sequencing-based method, an array based method and a PCR-based method.

According to an aspect of the invention, the expression levels of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 miRNAs are determined to obtain a pattern of expression levels.

According to an aspect of the invention, step a) comprises in step a) determining the expression level of the miRNAs: hsa-let-7f-5p, hsa-miR-1285-5p, hsa-miR-107, hsa-miR-103a-3p, hsa-miR-26b-3p, hsa-miR-26a-5p, hsa-miR-532-5p, hsa-miR-151a-3p, brain-mir-161, hsa-let-7d-3p, brain-mir-112, and hsa-miR-5010-3p.

The invention further relates to a kit for performing the methods of the invention, said kit comprising means for determining in said blood sample from said patient, an expression level of at least one miRNA selected from the group consisting of miRNAs with the sequence SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1 to SEQ ID NO 58, SEQ ID NO 60 to SEQ ID NO 64 and SEQ ID NO 66 to SEQ ID NO 170.

The means for determining the expression level of said at least one miRNA may comprise an oligonucleotide probe for detecting or amplifying said at least one miRNA, means for determining the expression level based on an array-based method, a PCR-based method, a sequencing-based method or any other suitable means for determining the expression level.

According to an aspect of the invention, the kit further comprises at least one reference pattern of expression levels for comparing with the expression level of the at least one miRNA from said sample. The reference pattern of expression may include at least one digital or numerical information and may be provided in any readable or electronically readable form, including, but not limited to printed form, electronically stored form on a computer readable medium, such as CD, smart card, or provided in downloadable form, e.g. in a computer network such as the internet.

The invention further relates to computer program product useful for performing the methods of the invention, comprising

- means for receiving data representing an expression level of at least one miRNA in a patient blood sample selected from the group consisting of miRNAs with the sequence SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1 to SEQ ID NO 58, SEQ ID NO 60 to SEQ ID NO 64 and SEQ ID NO 66 to SEQ ID NO 170,
- means for receiving data representing at least one reference pattern of expression levels for comparing with the expression level of the at least one miRNA from said sample,
- means for comparing said data representing the expression level of the at least one miRNA in a patient sample, and
- optionally means for determining a diagnosis of Alzheimer's Disease, a prediction of a risk of developing Alzheimer's Disease, or a prediction of an outcome of Alzheimer's Disease from the outcome of the comparison in step b).

The computer program product may be provided on a storable electronic medium, such as a solid state memory, disk, CD or other. It may be stored locally on a computer. It may be implemented as network-based program or application, including a web- or internet-based application. It may be implemented in a diagnostic device, such as an analyzer instrument. It may be operably connected to a device for outputting information, such as a display, printer or the like.

EXAMPLES

Additional details, features, characteristics and advantages of the object of the invention are further disclosed in the following description and figures of the respective examples, which, in an exemplary fashion, show preferred embodiments of the present invention. However, these examples should by no means be understood as to limit the scope of the invention.

The invention relates to methods for diagnosing Alheimer's Disease with miRNA markers.

Diagnosis of Alzheimer's Disease can be challenging in patients presenting with generally age-related syndromes such as forgetfulness. In particular, it is difficult to diagnose the earliest stages of disease. However, it would be particularly desirable to have a reliable diagnostic test for this stage of disease, as the chance of therapeutic and social intervention is better during this early disease stage.

Here, the abundance of miRNAs in blood samples of Alzheimer's Disease patients has been compared in an unbiased approach against healthy controls and patients suffering from other neuronal disorders. This approach involved a massive effort of sequencing miRNAs from samples and thus was open to the discovery of novel markers not yet described in the prior art. Further, the use of blood samples as a source of expression information of miRNA markers has several tangible advances which are not available in other sample sources such as serum or tissue, such as ease of sample procurement and handling, sample preparation, and robustness and consistency of expression patterns.

Materials and Methods

Patient Cohorts

The expression of miRNAs in peripheral blood of a total of 219 patients and healthy controls was determined, either by NGS or by qRT-PCR or both. Blood was obtained from patients with Alzheimer's Disease (AD) (n=106), patients with Mild Cognitive Impairement (MCI) (n=21), patients with Multiple Sclerosis (Clinically Isolated Syndrome, CIS) (n=17), patients with Parkinson's Disease (PD) (n=9), patients with Mild Depression (DEP) (n=15), Bipolar Disorder (BD) (n=15), Schizophrenia (Schiz) (n=14), and from healthy controls (n=22).

First, samples from AD patients (n=48), MCI patients (n=20) and healthy controls (n=22) were analyzed by Next-generation sequencing. For validation purposes the expression of single miRNAs was analyzed using qRT-PCR in the same samples as used for NGS, if enough RNA was available. The number of samples was further expanded by further samples from patients with AD, CIS, PD, DEP, BD, and Schiz, resulting in a total of 205 samples analyzed by qRT-PCR. In detail, a total of 95 samples from AD patients, 19 samples from MCI patients, 17 samples from CIS patients, 9 samples from PD patients, 15 samples from DEP patients, 15 samples from BD patients, 14 samples from Schiz patients, and 21 samples from healthy controls were analyzed.

RNA Isolation

Total RNA including miRNA was isolated using the PAXgene Blood miRNA Kit (Qiagen) following the manufacturer's recommendations. Isolated RNA was stored at −80° C. RNA integrity was analyzed using Bioanalyzer 2100 (Agilent) and concentration and purity were measured using NanoDrop 2000 (Thermo Scientific). A total of four samples (three controls and one RRMS) failed the quality criteria and were excluded from the study.

Library Preparation and Next-Generation Sequencing

For the library preparation, 200 ng of total RNA was used per sample, as determined with a RNA 6000 Nano Chip on the Bioanalyzer 2100 (Agilent). Preparation was performed following the protocol of the TruSeq Small RNA Sample Prep Kit (Illumina). Concentration of the ready prepped libraries was measured on the Bioanalyzer using the DNA 1000 Chip. Libraries were then pooled in batches of six samples in equal amounts and clustered with a concentration of 9 pmol in one lane each of a single read flowcell using the cBot (Illumina). Sequencing of 50 cycles was performed on a HiSeq 2000 (Illumina). Demultiplexing of the raw sequencing data and generation of the fastq files was done using CASAVA v.1.8.2.

NGS Data Analysis

The raw illumina reads were first preprocessed by cutting the 3' adapter sequence using the programm fastx_clipper from the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Reads shorter than 18 nts after clipping were removed. The remaining reads are reduced to unique reads and their frequency per sample to make the mapping steps more time efficient. For the remaining steps, we used the miRDeep2 pipeline. These steps consist of mapping the reads against the genome (hg19), mapping the reads against miRNA precursor sequences from mirbase release v18, summarizing the counts for the samples, and the prediction of novel miRNAs. Since the miRDeep2 pipeline predicts novel miRNAs per sample, the miRNAs were merged afterwards as follows: first, the novel miRNAs per sample that have a signal-to-noise ratio of more than 10 were extracted. Subsequently, only those novel miRNAs that are located on the same chromosome were merged, and both their mature forms share an overlap of at least 11 nucleotides.

Quantitative Real Time-PCR (qRT-PCR)

Out of the NGS results 7 miRNAs were selected that were deregulated in both, the comparison between patients with Alzheimer's Disease and patients with Mild Cognitive Impairment, and the comparison between patients with Alzheimer's Disease and healthy individuals. Five of the seven miRNAs, namely hsa-miR-5010-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-let-7d-3p, and hsa-miR-532-5p were already known mature miRNAs included in miRBase, two miRNAs, namely brain-mir-112 and brain-mir-161, were newly identified and not yet included in miRBase. As endogenous control the small nuclear RNA RNU48 as used.

The miScript PCR System (Qiagen) was used for reverse transcription and qRT-PCR. A total of 200 ng RNA was converted into cDNA using the miScript Reverse Transcription Kit according to the manufacturers' protocol. For each RNA we additionally prepared 5 μl reactions containing 200 ng RNA and 4 μl of the 5× miScript RT Buffer but no miScript Reverse Transcriptase Mix, as negative control for the reverse transcription (RT− control). The qRT-PCR was performed with the miScript SYBR® Green PCR Kit in a total volume of 20 μl per reaction containing 1 μl cDNA according to the manufacturers' protocol. For each miScript Primer Assay we additionally prepared a PCR negative-control with water instead of cDNA (non-template control, NTC).

Bioinformatics Analysis

First the read counts were normalized using standard quantile normalization. All miRNAs with less than 50 read counts were excluded from further considerations. Next, we calculated for each miRNA the area under the receiver operator characteristic curve (AUC), the fold-change, and the significance value (p-value) using t-tests. All significance values were adjusted for multiple testing using the Benjamini Hochberg approach. The bioinformatics analyses have been carried out using the freely available tool. R. Furthermore, we carried out a miRNA enrichment analysis using the TAM tool (http://202.38.126.151/hmdd/tools/tam.html).

Computing Combined Scores

Briefly, to compute a combined expression score for n up-regulated markers and m down-regulated markers the difference d between the expression value $x_{(a)}$ of a patient a and the average expression value of all controls μ is determined. For down-regulated markers, the difference can be multiplied by (−1), thus yielding a positive value. The differences for n markers can be added up to yield a combined score Z, such that

$$Z_{(a)}=\Sigma d_{(1\text{-}n)}(\text{upregulated})+\Sigma(-1)d_{(1\text{-}m)}(\text{down-regulated})$$

Wherein

$$d=x_{(a)}-\mu$$

To make combined scores between different marker scores comparable (e.g. to compare a (n+m)=7 marker score against a (n+m)=12 marker score, the combined score can be divided by (n+m):

$$Z\text{comp}=1/(n+m)(\Sigma d_{(1\text{-}n)}(\text{upregulated})+\Sigma(-1)d_{(1\text{-}m)}(\text{down-regulated}))$$

Other factors can be applied to the individual summands d of the combined score or the combined score Z as a whole.

Results

Screening Using High-Throughput Sequencing

To detect potential Alzheimer biomarkers a high-throughput sequencing of n=22 controls samples (C), n=48 Alzheimer patient (AD) samples and n=20 Mild Cognitive Impairment (MCI) samples was carried out. Precisely, Illumina HiSeq 2000 sequencing and multiplexed 8 samples on each sequencing lane was carried out. Thereby, 1150 of all human mature miRNAs in at least a single sample could be detected.

TABLE 1

Patient Cohorts

Disease	Cohort Size Screening	Cohort Size Replication
Controls	22	21
Alzheimer (US)	48	86
Alzheimer (GER)	0	9
Parkinson Disease	0	9
Mild Cognitive Impairment	20	18
Schizophrenia	0	14
Bipolar disease	0	15
Multiple Sclerosis (CIS)	0	17
Depression	0	15
SUM	90	204

The most abundant miRNAs were hsa-miR-486-5p with an average read-count of 13,886,676 and a total of 1.2 billion reads mapping to this miRNA, hsa-miR-92a-3p with an average of 575,359 reads and a total of 52 million reads mapping to this miRNA and miR-451a with an average of 135,012 reads and a total of 12 million reads mapping to this miRNA. The distribution of reads mapping to the three most abundant and all other miRNAs is shown in FIG. **1** (left pie chart). Additionally, 548 novel mature miRNA candidates were detected that have been previously not present in the

Sanger miRBase. These miRNA candidates have generally however been much less abundant as compared to the known human miRNAs. The most abundant one, denoted as brain-miR-314 was detected on average with 3,587 reads per sample and a total of 322,868 reads. Second highest expressed miRNA, brain-miR-247 was present on average with 3,112 and with a total of 280,115 reads, third most abundant miRNA brain-miR-12 at an average of 2,630 and a total of 236,728 reads. In the list of all, novel and known miRNAs, brain-miR-314 would be ranked on position 37, i.e., 36 known human miRNAs were more abundant than the highest abundant novel one. While a total of 1.4 Bn reads mapped to the known miRNAs, only 2.3 Mn mapped to the novel miRNA candidates. This relation shows that a very high sequencing capacity is required to reach the sensitivity in order to detect rare variants of novel miRNAs in human blood samples. Interestingly, as the right pie chart in FIG. **1** denotes, the candidate miRNAs are much more equally distributed as compared to the known ones, where the most abundant miRNA was responsible for 91% of all reads.

To detect potential biomarker candidates two-tailed t-tests and adjusted the significance values for multiple testing using Benjamini Hochberg adjustment were computed. All markers with adjusted significance values below 0.05 were considered statistically significant. Additionally, the area under the receiver operator characteristics curve (AUC) was computed to understand the specificity and sensitivity of miRNAs for Alzheimer diagnosis. Altogether, 170 significantly dys-regulated miRNAs we detected, 55 markers were significantly down-regulated in Alzheimer, while 115 were significantly up-regulated. A list of the respective 170 markers is presented in Supplemental Table 1 a and b. These 170 miRNA markers have the corresponding sequences SEQ ID NO 1 to SEQ ID NO 170 in the attached sequence protocol.

A list of all miRNA molecules described herein is given in Supplemental Table 4 containing an overview of the miRNA markers, including sequence information.

It is noted that the mature miRNa originate from miRNA precursor molecules of length of around 120 bases. Several examples exists where the miRNA precursors vary from each other while the subset of the around 20 bases belonging to the mature miRNA are identical. Thus, novel mature miRNAs can have the same sequence but different SEQ ID NO identifiers.

MiRNA markers are denoted by their common name (e.g. has-miR-144-5p or hsa-let 7f-5p) and are searchable in publically available databases. In this invention there are also described novel miRNA markers which have been named with names beginning with the prefix "brain-miR". They are listed in supplemental table 2 with their sequence and their SEQ ID NO according to the sequence protocol.

The ROC curves for the most up-regulated marker (hsa-miR-30d-5p with p-value of $8*10^{-9}$) as well as the most down-regulated marker (hsa-miR-144-5p with p-value of $1.5*10^{-5}$) are presented in FIGS. **2** and **3**, where the high AUC value indicates that already one single miRNA might have sufficient power to differentiate between cases and controls. Both miRNAs have however already been describe with many other human pathologies including different neoplasms and thus are non-specific for AD. Remarkably, the set of significant biomarkers also contained also 58 miRNAs that had so far not been reported, which have been designated with miRNA Names beginning with. Of these, only 10 were down-regulated while the majority of 48 miRNAs was highly up-regulated in AD.

To understand whether the detected biomarkers are also dys-regulated in MCI patients t-tests and AUC values for the comparison of healthy controls versus MCI were likewise computed. Here, ten markers remained statistically significant following adjustment for multiple testing. Of these, 8 were down-while 2 were up-regulated in MCI patients. Notably, 9 of them have been likewise significantly dys-regulated in MCI patients, namely hsa-miR-29c-3p, hsa-miR-29a-3p, hsa-let-7e-5p, hsa-let-7a-5p, hsa-let-7f-5p, hsa-miR-29b-3p, hsa-miR-98, hsa-miR-425-5p and hsa-miR-181a-2-3p. Only miRNA hsa-miR-223-3p was just significant in MCI patients while not in AD patients. A full list of all MCI biomarkers, identified as SEQ ID NO 171-235 in the attached sequence listing is presented in Supplemental Table 3. It is noted that mature miRNA originate from miRNA precursor molecules of length of around 120 bases. Several examples exists where the miRNA precursors vary from each other while the subset of the around 20 bases belonging to the mature miRNA are identical. Thus, novel mature miRNAs can have the same sequence but different identifiers.

Besides single markers, combinations of multiple markers have demonstrated a potential to improve the diagnostic accuracy. To test this hypothesis, a standard machine learning approach was applied. In a cross-validation loop, the markers with lowest significance values were stepwise added and repeatedly radial basis function support vector machines were carried out. The accuracy, specificity and sensitivity depend on the number of biomarkers are presented in FIG. **4**. As shown there, accuracy, specificity and sensitivity increase up to a signature number of 250 miRNAs and then converge to 90%. However, this set of miRNAs contains a significant amount of redundant biomarkers, i.e., markers that have almost identical information content to each other and are highly correlated such that even significantly smaller sets of markers can be expected to perform highly accurate distinction in Alzheimer samples and controls. We selected a signature of just 7 markers, namely the up-regulated miRNAs brain-mir-112, brain-mir-161, hsa-let-7d-3p and hsa-miR-5010-3p as well as the down-regulated markers hsa-miR-103a-3p, hsa-miR-107 and hsa-miR-532-5p. To combine the values of the 7 miRNAs in one score we calculated the average z-score as detailed in the Material & Methods section. While we reached averaged values of 0.087 and standard deviation of 0.72 for the controls and average values of 0.22 and standard deviation of 0.74 for the MCI patients, AD patients reached a much higher score of 0.63 at a standard deviation of 0.64. Thus, the Alzheimer patients have significantly higher scores as controls, indicated by the two-tailed t-test p-value of 0.025. These numbers are detailed as bar-chart in FIG. **5**. The ROC curve for the signature showing an AUC of 84.3% with 95% CI of 75.3%-93.2% is presented in FIG. **6**.

A further signature of 12 markers with limited cross-correlation was selected, including the most strongly dys-regulated markers that are less frequently dys-regulated in other diseases and show a potential to separate AD also from MCI. More precisely, this selected signature contains the up-regulated miRNAs brain-mir-112, brain-mir-161, hsa-let-7d-3p, hsa-miR-5010-3p, hsa-miR-26b-3p, hsa-miR-26a-5p, hsa-miR-1285-5p, and hsa-miR-151a-3p as well as the down-regulated markers hsa-miR-103a-3p, hsa-miR-107, hsa-miR-532-5p, and hsa-let-7f-5p. To combine the values of the 12 miRNAs in one score the combined score was computed as discussed above. While averaged values of 0 and standard deviation of 0.39 for the controls were reached and average values of 0.32 and standard deviation of 0.5 for the MCI patients were reached, AD patients reached a much higher score of 0.93 at a standard deviation of 0.54. Thus, the Alzheimer patients have significantly higher scores as controls, indicated by the two-tailed t-test p-value of $3.7*10^{-11}$ in case of AD versus C as well as $6*10^{-5}$ in case of AD versus MCI. In addition we computed the same scores for a set of 15 MS samples, showing a likewise decreased score of 0.1 at standard deviation of 0.34.

Biological Relevance of miRNAs for AD

To understand the biological function of the dys-regulated miRNAs better a miRNA enrichment analysis for the up- and down-regulated miRNAs was applied (Ming Lu, Bing Shi, Juan Wang, Qun Cao and Qinghua Cui. TAM: A method for enrichment and depletion analysis of a microRNA category in a list of microRNAs. BMC Bioinformatics 2010, 11:419 (9 Aug. 2010). The results of this analysis are detailed in Table 2. Altogether, for the 55 down-regulated miRNAs 11 significant categories after adjustment for multiple testing were detected while for the 115 up-regulated just a single category remained significant, the miR-30 family with 5 members being up-regulated. In contrast, for the down-regulated miRNAs 7 miRNAs of the let-7 family were found being significant. In addition, the set contained also 8 miRNAs belonging to anti-cell proliferation and 13 tumor suppressors. Finally, we were able to show that the down-regulated miRNAs correlate to 8 diseases, including Alzheimer. Here, we found 5 miRNAs being relevant, including hsa-miR-17, hsa-miR-29a, hsa-miR-29b, hsa-miR-106b and hsa-miR-107.

TABLE 2

Regulated Pathways and categories

	down		up	
Term	Count	p-value	Count	p-value
anti-cell proliferation	8	4.60-3	n.s.	n.s.
miRNA tumor suppressors	13	6.71-3	n.s.	n.s.
let-7 family	7	7.00-3	n.s.	n.s.
Digestive System Neoplasms	6	0.0144	n.s.	n.s.
Pituitary Neoplasms	7	0.0168	n.s.	n.s.
Lymphoma, Primary Effusion	7	0.0201	n.s.	n.s.
Sarcoma, Kaposi	7	0.021	n.s.	n.s.
Carcinoma, Non-Small Cell Lung	6	0.027	n.s.	n.s.
Neoplasms	14	0.028	n.s.	n.s.
Colonic Neoplasms	12	0.0388	n.s.	n.s.
Alzheimer Disease	5	0.0433	n.s.	n.s.
mir-30 family	n.s.	n.s.	5	8.95-3

Validation of Signature by q-RT-PCR

In order to transfer the signature to clinical routine settings it is essential that the proposed in-vitro diagnostic test can be applied in molecular diagnostic labs in reasonable time using standard equipment. To this end, qRT-PCR represents a suitable solution to replicate and validate our AD signature using this approach. In addition to measure just controls, AD and MCI patients, a wide range of other neurological disorders were also included. For AD, besides the US cohort also a set of samples collected in Germany were included. The full overview on measured samples is provided in Table 1.

First, the fold quotients of the initial screening cohort were compared and analyzed by next-generation sequencing and this was compared to the performance of the same miRNAs by qRT-PCR. As the scatter-plot in FIG. **7** presents, all miRNAs have been dys-regulated in the same direction by both approaches and in both cohorts, indicating a very high degree of concordance between screening and validation study. As for the next generation sequencing screening approach AUC values were calculated for the validation qRT-PCR cohort. The best single miRNA was miR-5010-3p with an AUC of 84.5% (AUC of screening: 75.5%). On average, the 7 miRNAs reached an AUC value of 71%, indicating the high diagnostic information content. Next, the question was addressed whether the combination of the 7 miRNAs can further improve the diagnosis of AD. The same z-scored based approach was applied.

While averaged values of 0.087 and standard deviation of 0.72 for the controls and average values of 0.22 and standard deviation of 0.74 were reached for the MCI patients, AD patients reached a much higher score of 0.63 at a standard deviation of 0.64.

For controls an average value of 0 (screening: −0.087) at a standard deviation of 0.34 (screening: 0.72) was obtained, while for AD patients, the score was as high as 0.7 (screening 0.63) at standard deviation of 0.45 (screening: 0.64). Thus, AD patients have significantly higher values as compared to controls since the 2-tailed t-test p-value is as low as 1.3*10-9 (screening 0.025). The z-scores are presented as bar-diagram in FIG. **10**. Here, it can be clearly seen that especially the standard deviations are much smaller for the qRT-PCR based validation cohort.

Scores of Other Neurological Disorders

Next the question was asked whether a cohort of other neurological disorders shows likewise significant deviations to controls. As detailed in Table 1 we measured a second cohort of Alzheimer patients, Parkinson disease, mild cognitive impairment, schizophrenia, bipolar disorder, multiple sclerosis (CIS) depression patients for the signature of 7 miRNAs. In FIG. **11**, the bar diagrams for all diseases and all miRNAs are present. Here, the Alzheimer patients score is set to 0, as described earlier we have four down- and three up-regulated miRNAs for the controls. For mild cognitive impairment patients the same four miRNAs were down- and the same three miRNAs were up-regulated, providing strong evidence that the MCI signature is much closer to controls as compared to AD. For CIS patients only two miRNAs were down-regulated, while the third one was not dys-regulated and the remaining three were strongly up-regulated. For Parkinson disease, the first 5 miRNAs were down-while the remaining two were strongly up-regulated. For Schizophrenia and Bipolar Disease, almost all miRNAs were strongly up-regulated, in contrast, for Depression all miRNAs were significantly down-regulated. In summary, the results promise that AD can not only be distinguished from controls but also very well from other neurological disorders. Of course the same z-score based approach can be applied as for the Alzheimer and control patients in order to get an overall score for each cohort.

Further significant signatures of miRNA for differentiating between AD and controls have been found:

hsa-miR-1285-5p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p,

hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,

hsa-miR-3127-3p hsa-miR-3157-3p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,

hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-151a-3p hsa-miR-144-5p,

hsa-miR-1285-5p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7a-5p,

hsa-miR-5001-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,

hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p, hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,

hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-miR-144-5p hsa-let-7a-5p,

hsa-miR-1285-5p brain-mir-112 hsa-miR-425-5p hsa-miR-151a-3p hsa-miR-144-5p,

hsa-miR-5001-3p hsa-miR-1285-5p brain-mir-112 hsa-miR-151a-3p hsa-let-7f-5p,

hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-148b-5p hsa-miR-144-5p hsa-let-7f-5p,

hsa-miR-1285-5p hsa-miR-3157-3p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,

hsa-miR-5001-3p hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p,

brain-mir-431 hsa-miR-1285-5p hsa-miR-3157-3p hsa-miR-151a-3p hsa-miR-144-5p,

hsa-miR-3127-3p hsa-miR-1285-5p brain-mir-112 hsa-miR-425-5p hsa-miR-151a-3p,

hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-miR-144-5p hsa-let-7f-5p,

hsa-miR-550a-5p hsa-miR-1285-5p brain-mir-112 hsa-miR-151a-3p hsa-let-7f-5p,

hsa-miR-1285-5p brain-mir-112 hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p, and

hsa-miR-5001-3p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p.

These are further preferred combinations for classifying a sample of a patient suffering from or at risk of developing Alzheimer's Disease or diagnosing AD, or predicting an outcome of AD (ca. Table 3)

TABLE 3

Further preferred signatures for diagnosing AD.

Signature	AUC	Mean AD	mean Control	mean MCI	mean AD replication	miRNA 1	miRNA 2	miRNA 3	miRNA 4	miRNA 5
sig #1	0.011	1.123	−0.019	0.557	1.190	hsa-miR-1285-5p	brain-mir-112	hsa-miR-5010-3p	hsa-miR-151a-3p	hsa-let-7f-5p
sig #2	0.011	1.054	−0.012	0.549	1.281	hsa-miR-3127-3p	hsa-miR-1285-5p	hsa-miR-425-5p	hsa-miR-148b-5p	hsa-miR-144-5p
sig #3	0.015	1.101	−0.028	0.454	1.137	hsa-miR-3127-3p	hsa-miR-3157-3p	hsa-miR-148b-5p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #4	0.015	1.097	−0.015	0.663	1.325	hsa-miR-3127-3p	hsa-miR-1285-5p	hsa-miR-425-5p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #5	0.016	1.111	−0.020	0.561	1.187	hsa-miR-1285-5p	brain-mir-112	hsa-miR-5010-3p	hsa-miR-151a-3p	hsa-let-7a-5p
sig #6	0.018	1.078	0.003	0.515	1.318	hsa-miR-5001-3p	hsa-miR-1285-5p	hsa-miR-425-5p	hsa-miR-148b-5p	hsa-miR-144-5p
sig #7	0.020	1.097	−0.015	0.490	1.140	hsa-miR-3127-3p	hsa-miR-1285-5p	hsa-miR-148b-5p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #8	0.020	1.062	−0.010	0.493	1.299	hsa-miR-1285-5p	hsa-miR-5010-3p	hsa-miR-425-5p	hsa-miR-148b-5p	hsa-miR-144-5p
sig #9	0.021	1.152	0.002	0.645	1.332	hsa-miR-1285-5p	hsa-miR-5010-3p	hsa-miR-151a-3p	hsa-miR-144-5p	hsa-let-7a-5p
sig #10	0.021	1.139	−0.014	0.614	1.217	hsa-miR-1285-5p	brain-mir-112	hsa-miR-425-5p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #11	0.021	1.139	−0.006	0.579	1.209	hsa-miR-5001-3p	hsa-miR-1285-5p	brain-mir-112	hsa-miR-151a-3p	hsa-let-7f-5p
sig #12	0.021	1.120	0.006	0.527	1.291	hsa-miR-1285-5p	hsa-miR-5010-3p	hsa-miR-148b-5p	hsa-miR-144-5p	hsa-let-7f-5p
sig #13	0.021	1.111	−0.015	0.400	1.031	hsa-miR-1285-5p	hsa-miR-3157-3p	hsa-miR-148b-5p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #14	0.021	1.105	−0.004	0.572	1.335	hsa-miR-5001-3p	hsa-miR-1285-5p	hsa-miR-5010-3p	hsa-miR-151a-3p	hsa-let-7f-5p
sig #15	0.021	1.098	−0.021	0.492	0.767	brain-mir-431	hsa-miR-1285-5p	hsa-miR-3157-3p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #16	0.021	1.056	−0.038	0.579	1.180	hsa-miR-3127-3p	hsa-miR-1285-5p	brain-mir-112	hsa-miR-425-5p	hsa-miR-151a-3p
sig #17	0.022	1.164	0.003	0.641	1.335	hsa-miR-1285-5p	hsa-miR-5010-3p	hsa-miR-151a-3p	hsa-miR-144-5p	hsa-let-7f-5p
sig #18	0.022	1.140	−0.015	0.691	0.649	hsa-miR-550a-5p	hsa-miR-1285-5p	brain-mir-112	hsa-miR-151a-3p	hsa-let-7f-5p
sig #19	0.022	1.140	−0.014	0.441	1.033	hsa-miR-1285-5p	brain-mir-112	hsa-miR-148b-5p	hsa-miR-151a-3p	hsa-miR-144-5p
sig #20	0.022	1.137	−0.016	0.576	1.333	hsa-miR-5001-3p	brain-mir-112	hsa-miR-5010-3p	hsa-miR-151a-3p	hsa-let-7f-5p

SUPPLEMENTAL TABLE 1 a

Significantly down-regulated miRNAs in AD vs. controls.

SEQ ID NO	miRNA	median AD	median Control	t-test p-value single	AUC
1	hsa-miR-144-5p	179.3082706767	913.7744360902	8.76E−08	0.0928030303
2	hsa-let-7f-5p	8334.1804511278	12867.954887218	7.60E−07	0.0710227273
3	hsa-let-7e-5p	4971.7669172932	8212.9360902256	8.58E−07	0.1382575758
4	hsa-let-7a-5p	8868.4511278196	15370.052631579	4.43E−06	0.0880681818
5	hsa-miR-107	2433.0413533835	4822.3984962406	1.82E−05	0.203125
6	hsa-let-7g-5p	1352.3684210526	3403.3759398496	2.69E−05	0.1259469697
7	hsa-miR-103a-3p	2810.8458646617	5290.1278195489	3.89E−05	0.2088068182
8	hsa-miR-98	106.0864661654	217.3533834586	4.28E−05	0.1515151515
9	hsa-miR-29c-3p	40.8327067669	74.8402255639	6.87E−05	0.1922348485
10	hsa-miR-101-3p	56.1090225564	417.7105263158	0.0001143344	0.1463068182
11	hsa-miR-548h-5p	3.6296992481	10.0845864662	0.000139166	0.1770833333
12	hsa-miR-106b-3p	1685.5864661654	2614.7518796993	0.0001453506	0.2097537879
13	hsa-miR-15a-5p	598.484962406	1472.9962406015	0.0001554169	0.1557765152
14	hsa-miR-548g-5p	3.0338345865	9.6296992481	0.0002158917	0.1979166667
15	hsa-miR-548ar-5p	3.0338345865	9.6296992481	0.0002158917	0.1979166667
16	hsa-miR-548x-5p	3.0338345865	9.6296992481	0.0002158917	0.1979166667
17	hsa-miR-548aj-5p	3.0338345865	9.6296992481	0.0002158917	0.1979166667
18	hsa-let-7c	6147.5714285714	11249.522556391	0.0002796738	0.1661931818
19	brain-mir-394	2.8026315789	7.8364661654	0.0003164566	0.2059659091
20	hsa-miR-1294	8.765037594	25.9379699248	0.0003239282	0.2026515152
21	brain-mir-170	2.8026315789	7.8364661654	0.0003241053	0.2069128788
22	hsa-miR-199a-3p	4.0883458647	12.454887218	0.0003438893	0.1595643939
23	brain-mir-149	2.8157894737	7.8364661654	0.000344696	0.2097537879
24	brain-mir-151	2.8157894737	7.8364661654	0.000344696	0.2097537879
25	brain-mir-370	178.4586466165	778.2894736842	0.0003625216	0.1553030303
26	hsa-miR-199b-3p	4.0883458647	12.6259398496	0.0003732986	0.1652462121
27	brain-mir-333	2.8026315789	7.8364661654	0.0004122695	0.2069128788
28	hsa-miR-628-3p	2.954887218	7.1165413534	0.0004263003	0.2320075758
29	hsa-miR-190a	1.0488721805	5.5977443609	0.0004511324	0.1837121212
30	hsa-miR-29b-3p	11.8120300752	23.6503759398	0.0005076275	0.228219697
31	hsa-miR-660-5p	20.2537593985	72.9116541353	0.0006111848	0.1766098485
32	hsa-miR-143-3p	81.0676691729	168.0714285714	0.0006300042	0.2277462121
33	hsa-miR-548av-5p	3.2593984962	9.6541353383	0.0006819514	0.2196969697
34	hsa-miR-548k	3.2593984962	9.6541353383	0.0006819514	0.2196969697
35	hsa-miR-29a-3p	43.6917293233	74.4943609023	0.0008592211	0.2414772727
36	hsa-miR-548i	0.1992481203	1.1090225564	0.0009595931	0.1482007576
37	hsa-miR-17-3p	32.5563909774	80.7781954887	0.0015924698	0.1410984848
38	brain-mir-398	10.0338345865	29.5526315789	0.0016819805	0.1964962121
39	hsa-miR-148a-3p	274.1748120301	845.5263157895	0.001762298	0.1586174242
40	hsa-miR-126-3p	39.045112782	108.8195488722	0.0028031688	0.2135416667
41	brain-mir-150	6.4266917293	19.4812030075	0.0034501841	0.2201704545
42	hsa-let-7i-5p	2907.4210526316	6027.2030075188	0.0034616244	0.2059659091
43	hsa-miR-33b-5p	0.227443609	2.1240601504	0.0035364268	0.2580492424
44	hsa-miR-3200-3p	16.765037594	23.5037593985	0.0045456431	0.3233901515
45	hsa-miR-548o-5p	0.3834586466	1.7593984962	0.0047156877	0.2831439394
46	hsa-miR-152	11.2142857143	22.2312030075	0.0052379113	0.1983901515
47	hsa-miR-548am-5p	0.4887218045	1.7593984962	0.0053080221	0.2878787879
48	hsa-miR-548au-5p	0.4887218045	1.7593984962	0.0053080221	0.2878787879
49	hsa-miR-548c-5p	0.4887218045	1.7593984962	0.0053080221	0.2878787879
50	brain-mir-248S	0.2443609023	0.9285714286	0.0065438684	0.2547348485
51	hsa-miR-215	2042.3909774436	2997.969924812	0.008661199	0.3072916667
52	hsa-miR-340-5p	7.5977443609	21.984962406	0.0088183152	0.271780303
53	hsa-miR-1301	6.7330827068	9.5488721805	0.0089721175	0.2845643939
54	brain-mir-145	13.9511278195	17.7556390977	0.008979579	0.3143939394
55	hsa-miR-504	0.3834586466	1.8026315789	0.0093874443	0.3697916667

SUPPLEMENTAL TABLE 1 b

Significantly up-regulated miRNAs in AD vs. controls.

SEQ ID NO	miRNA	median AD	median Control	t-test p-value single	AUC
56	hsa-miR-30d-5p	11759.6691729323	7038.4962406015	9.25E−12	0.8863636364
57	hsa-miR-4781-3p	20.1597744361	10.0714285714	8.76E−10	0.8726325758
58	hsa-miR-151a-3p	3303.037593985	1892.6616541353	3.49E−08	0.8645833333
59	brain-mir-112	10.242481203	3.2687969925	4.77E−08	0.8735795455
60	hsa-miR-28-3p	1009.6466165414	537.7894736842	1.17E−07	0.7845643939
61	hsa-miR-26b-3p	73.6240601504	29.2105263158	1.18E−07	0.8333333333
62	hsa-miR-1468	80.1296992481	34.6466165414	9.00E−07	0.7732007576
63	hsa-miR-128	1204.3533834587	761.5676691729	9.93E−07	0.8238636364
64	hsa-miR-550a-5p	61.6052631579	39.4135338346	1.93E−06	0.8143939394
65	hsa-miR-5010-3p	134.5263157895	77.8684210526	2.52E−06	0.8191287879
66	hsa-miR-148b-5p	24.1278195489	12.8928571429	2.85E−06	0.8096590909
67	brain-mir-395	7.8759398496	4.3233082707	3.18E−06	0.7935606061
68	brain-mir-308	7.8759398496	4.3233082707	3.18E−06	0.7935606061
69	hsa-miR-1285-5p	7.0695488722	3.2030075188	3.47E−06	0.7954545455
70	hsa-miR-5001-3p	14.8796992481	7.0714285714	4.41E−06	0.8077651515
71	hsa-miR-3127-3p	5.8421052632	2.4718045113	5.13E−06	0.7883522727
72	hsa-miR-3157-3p	7.3778195489	3.1616541353	7.70E−06	0.8181818182
73	brain-mir-431	6.2462406015	2.9436090226	8.30E−06	0.7869318182
74	hsa-miR-550a-3-5p	53.4661654135	31.9680451128	8.51E−06	0.7987689394
75	hsa-miR-361-5p	51.6973684211	28.5733082707	1.18E−05	0.7940340909
76	brain-mir-83	160.5808270677	95.3872180451	1.37E−05	0.7367424242
77	hsa-miR-589-5p	305.6390977444	227.015037594	1.54E−05	0.7698863636
78	hsa-miR-425-5p	5290.1278195489	2907.4210526316	1.61E−05	0.8020833333
79	hsa-miR-30a-5p	10739.3759398496	7557.4210526316	2.66E−05	0.7826704545
80	brain-mir-79	3.5206766917	1.3026315789	2.85E−05	0.7552083333
81	brain-mir-80	3.5206766917	1.3026315789	2.85E−05	0.7552083333
82	hsa-miR-330-5p	10.7312030075	6.3402255639	3.46E−05	0.7722537879
83	hsa-miR-186-5p	4206.2932330827	2433.0413533835	3.46E−05	0.775094697
84	brain-mir-390	5.4191729323	3.1428571429	3.85E−05	0.7618371212
85	hsa-let-7d-3p	391.4060150376	208.9398496241	3.95E−05	0.7069128788
86	hsa-miR-328	396.6992481203	204.6898496241	4.08E−05	0.7168560606
87	hsa-miR-30c-5p	3195.7781954887	1563.7631578947	4.79E−05	0.7769886364
88	brain-mir-200	30.3740601504	15.8233082707	5.41E−05	0.7665719697
89	hsa-miR-363-3p	6371.4285714286	4971.7669172932	5.51E−05	0.7552083333
90	hsa-miR-339-3p	125.3120300752	87.8345864662	5.67E−05	0.7471590909
91	brain-mir-114	1009.6466165414	543.5526315789	5.76E−05	0.6856060606
92	hsa-miR-942	512.7142857143	306.2894736842	6.12E−05	0.6851325758
93	hsa-miR-345-5p	470.6090225564	317.9210526316	6.17E−05	0.7481060606
94	brain-mir-247	2997.969924812	1634.6879699248	7.23E−05	0.7315340909
95	hsa-miR-4742-3p	43.2030075188	27.6635338346	7.99E−05	0.7201704545
96	brain-mir-314	3614.8045112782	2124.5751879699	8.13E−05	0.7324810606
97	brain-mir-12	2433.0413533835	1370.5338345865	9.13E−05	0.7220643939
98	brain-mir-232	75.0733082707	39.9285714286	9.70E−05	0.6799242424
99	brain-mir-424S	4.8571428571	2.1503759398	0.0001134253	0.7608901515
100	brain-mir-219	28.5751879699	15.7819548872	0.0001441433	0.7736742424
101	hsa-miR-10a-5p	827.0977443609	443.9586466165	0.0001696328	0.7334280303
102	hsa-miR-3605-3p	280.9135338346	187.6466165414	0.0001817728	0.6837121212
103	brain-mir-52	9.2406015038	4.6503759398	0.0002065404	0.7817234848
104	brain-mir-53	6.7462406015	3.8909774436	0.0002097674	0.7604166667
105	hsa-miR-3157-5p	0.3721804511	0.1240601504	0.0002118311	0.7277462121
106	brain-mir-41S	10.5733082707	5.9191729323	0.0002570966	0.7803030303
107	brain-mir-201	15.4248120301	9.5469924812	0.000293033	0.7291666667
108	hsa-miR-5006-3p	2.5921052632	1.4210526316	0.0003127522	0.743844697
109	hsa-miR-4659a-3p	7.2255639098	4.0977443609	0.0003606508	0.7447916667
110	brain-mir-279	10.1334586466	5.1541353383	0.000437069	0.6955492424
111	brain-mir-111	986.477443609	590.4022556391	0.0004713764	0.7504734848
112	brain-mir-88	2.3646616541	1.3778195489	0.0005681084	0.6912878788
113	brain-mir-251	1.8909774436	0.8458646617	0.0005688548	0.7296401515
114	hsa-miR-4435	51.0902255639	33.9661654135	0.0005693209	0.7230113636

SUPPLEMENTAL TABLE 1 b-continued

Significantly up-regulated miRNAs in AD vs. controls.

SEQ ID NO	miRNA	median AD	median Control	t-test p-value single	AUC
115	hsa-miR-5690	11.3984962406	7.5281954887	0.0005745024	0.7253787879
116	brain-mir-166	2.4210526316	1.0921052632	0.0006242931	0.7149621212
117	brain-mir-193	1.6127819549	0.8402255639	0.0006339444	0.7002840909
118	hsa-miR-625-5p	7.3590225564	4.3571428571	0.0006972852	0.7575757576
119	hsa-miR-10b-5p	683.6766917293	406.3007518797	0.0008299916	0.7168560606
120	brain-mir-299	3.9586466165	1.7857142857	0.000839426	0.7069128788
121	brain-mir-153	0.5751879699	0.1428571429	0.0008478946	0.6860795455
122	hsa-miR-758	0.5939849624	0.1240601504	0.0008889247	0.7589962121
123	hsa-miR-30a-3p	114.6278195489	67.3947368421	0.0009850641	0.7357954545
124	brain-mir-220	36.4530075188	24.4511278195	0.0010085027	0.7182765152
125	brain-mir-392	5.5695488722	3.1447368421	0.001117105	0.6586174242
126	brain-mir-102	34.0526315789	22.9229323308	0.0011430551	0.7571022727
127	hsa-let-7b-3p	47.2894736842	26.0338345865	0.0011483131	0.7471590909
128	hsa-miR-340-3p	23.6879699248	9.4248120301	0.0011789284	0.7651515152
129	hsa-miR-484	21682.0451127819	14260.5789473684	0.0012569269	0.7211174242
130	hsa-miR-30e-3p	169.3082706767	121.1917293233	0.0013440534	0.7381628788
131	brain-mir-72S	0.4436090226	0.1240601504	0.0014225572	0.7348484848
132	hsa-miR-371b-5p	4.7142857143	2.2706766917	0.0014389281	0.7258522727
133	hsa-miR-5581-3p	2.3327067669	1.5620300752	0.0015546337	0.7064393939
134	brain-mir-399	19.1616541353	12.7706766917	0.0015845513	0.6619318182
135	brain-mir-403	4.1842105263	2.8364661654	0.0016408632	0.6695075758
136	brain-mir-73	21.1766917293	12.992481203	0.0016958209	0.6922348485
137	brain-mir-190	4.3233082707	2.3590225564	0.0020611484	0.6903409091
138	brain-mir-188	4.3233082707	2.3590225564	0.0020611484	0.6903409091
139	brain-mir-189	4.3233082707	2.3590225564	0.0020611484	0.6903409091
140	brain-mir-192	4.3233082707	2.3590225564	0.0020611484	0.6903409091
141	brain-mir-311	382.2819548872	266.9248120301	0.0022861501	0.6373106061
142	brain-mir-161	17.4887218045	10.5	0.0024185375	0.7424242424
143	hsa-miR-3074-5p	24.015037594	15.7105263158	0.002419588	0.740530303
144	hsa-miR-30b-5p	443.9586466165	292.2105263158	0.0024240637	0.712594697
145	hsa-miR-576-5p	291.3834586466	207.484962406	0.0024324256	0.7215909091
146	brain-mir-23	16.2218045113	11.3665413534	0.0024712736	0.71875
147	hsa-miR-943	2.0789473684	1.3984962406	0.0025973005	0.6903409091
148	brain-mir-351	0.272556391	0.1278195489	0.0026770024	0.6439393939
149	hsa-miR-4772-3p	1.0601503759	0.219924812	0.0030588227	0.6884469697
150	brain-mir-319	4.954887218	3.6860902256	0.0031658495	0.6912878788
151	hsa-miR-937	13.8984962406	8.4323308271	0.0032014572	0.6174242424
152	hsa-miR-181a-2-3p	222.4135338346	173.3458646617	0.0034658731	0.6770833333
153	hsa-miR-4755-5p	6.4661654135	4.0789473684	0.003589103	0.6590909091
154	hsa-miR-3909	7.7011278195	4.1691729323	0.0036634327	0.7466856061
155	hsa-miR-1260b	548	436.8947368421	0.0037982461	0.640625
156	brain-mir-293	3.4022556391	2.0056390977	0.0043533661	0.6879734848
157	brain-mir-160	13.1635338346	9.3646616541	0.0047314115	0.6496212121
158	hsa-miR-2110	37.5056390977	20.3082706767	0.0048976896	0.7755681818
159	hsa-miR-584-3p	1.6184210526	0.8289473684	0.0049666999	0.6401515152
160	brain-mir-129	1.2312030075	0.8139097744	0.0052865283	0.6557765152
161	hsa-miR-1280	2.8233082707	1.1860902256	0.0054091313	0.6519886364
162	hsa-miR-3180-5p	1.0939849624	0.515037594	0.0064691451	0.6557765152
163	hsa-miR-668	0.3289473684	0.1390977444	0.0064710752	0.640625
164	hsa-miR-4512	2.0112781955	0.787593985	0.0068965461	0.6638257576
165	hsa-miR-641	10.0902255639	7.5620300752	0.0069660105	0.6619318182
166	hsa-miR-1233	2.0601503759	0.9285714286	0.007463631	0.6586174242
167	hsa-miR-378a-5p	10.0263157895	5.4755639098	0.0075454956	0.7149621212
168	hsa-miR-26a-5p	5634.0676691729	4206.2932330827	0.007829731	0.6789772727
169	brain-mir-258	5.6973684211	0.8233082707	0.0079015891	0.7201704545
170	hsa-miR-1260a	553.045112782	456.4210526316	0.0091301492	0.6070075758

SUPPLEMENTAL TABLE 2

Newly discovered miRNA markers		
SEQ ID NO	miRNA	Sequence
126	brain-mir-102	UAUGGAGGUCUCUGUCUGGCU
111	brain-mir-111	CACUGCUAAAUUUGGCUGGCUU
59	brain-mir-112	AGCUCUGUCUGUGUCUCUAGG
91	brain-mir-114	CACUGCAACCUCUGCCUCCGGU
97	brain-mir-12	ACUCCCACUGCUUGACUUGACUAG
160	brain-mir-129	CAUGGUCCAUUUUGCUCUGCU
54	brain-mir-145	AAGCACUGCCUUUGAACCUGA
23	brain-mir-149	AAAAGUAAUCGCACUUUUUG
41	brain-mir-150	UGAGGUAGUAGGUGGUGUGC
24	brain-mir-151	AAAAGUAAUCGCACUUUUUG
121	brain-mir-153	CCUCUUCUCAGAACACUUCCUGG
157	brain-mir-160	CACUGCAACCUCUGCCUCC
142	brain-mir-161	CUUCGAAAGCGGCUUCGGCU
116	brain-mir-166	CUGGCUGCUUCCCUUGGUCU
21	brain-mir-170	AAAAGUAAUGGCAGUUUUUG
138	brain-mir-188	CCUGACCCCCAUGUCGCCUCUGU
139	brain-mir-189	CCUGACCCCCAUGUCGCCUCUGU
137	brain-mir-190	CCUGACCCCCAUGUCGCCUCUGU
140	brain-mir-192	CCUGACCCCCAUGUCGCCUCUGU
117	brain-mir-193	AUCCCUUUAUCUGUCCUCUAGG
88	brain-mir-200	UUCCUGGCUCUCUGUUGCACA
107	brain-mir-201	CACCCCACCAGUGCAGGCUG
100	brain-mir-219	UCAAGUGUCAUCUGUCCCUAGG
124	brain-mir-220	UCCGGAUCCGGCUCCGCGCCU
146	brain-mir-23	UUAGUGGCUCCCUCUGCCUGCA
98	brain-mir-232	UUGCUCUGCUCUCCCUUGUACU
94	brain-mir-247	ACGCCCACUGCUUCACUUGACUAG
50	brain-mir-248S	GGCGGCGGAGGCGGCGGUG
113	brain-mir-251	UGGCCCAAGACCUCAGACC

SUPPLEMENTAL TABLE 2-continued

Newly discovered miRNA markers		
SEQ ID NO	miRNA	Sequence
169	brain-mir-258	AUCCCACCCCUGCCCCCA
110	brain-mir-279	AUCCCACCGCUGCCACAC
156	brain-mir-293	UUGGUGAGGACCCCAAGCUCGG
120	brain-mir-299	CAUGCCACUGCACUCCAGCCU
68	brain-mir-308	CACUGCACUCCAGCCUGGGUGA
141	brain-mir-311	CACUGCAACCUCUGCCUCCCGA
96	brain-mir-314	ACUCCCACUGCUUCACUUGAUUAG
150	brain-mir-319	CUGCACUCCAGCCUGGGCGA
27	brain-mir-333	AAAAGUAAUCGCAGGUUUUG
148	brain-mir-351	UGUCUUGCUCUGUUGCCCAGGU
25	brain-mir-370	GGCUGGUCUGAUGGUAGUGGGUUA
84	brain-mir-390	ACUGCAACCUCCACCUCCUGGGU
125	brain-mir-392	CCCGCCUGUCUCUCUCUUGCA
19	brain-mir-394	AAAAGUAAUCGUAGUUUUUG
67	brain-mir-395	CACUGCACUCCAGCCUGGGUGA
38	brain-mir-398	GGCUGGUCCGAGUGCAGUGGUGUU
134	brain-mir-399	CACUGCAACCUCUGCCUCC
135	brain-mir-403	AAAGACUUCCUUCUCUCGCCU
106	brain-mir-41S	CCCCGCGCAGGUUCGAAUCCUG
99	brain-mir-424S	CACUGCACUCCAGCCUGGGUA
73	brain-mir-431	CUCGGCCUUUGCUCGCAGCACU
103	brain-mir-52	CUGCACUCCAGCCUGGGCGAC
104	brain-mir-53	CCCAGGACAGUUUCAGUGAUG
131	brain-mir-72S	GACCACACUCCAUCCUGGGC
136	brain-mir-73	UCCGGAUGUGCUGACCCCUGCG
80	brain-mir-79	CACUGCACUCCAGCCUGGCU
81	brain-mir-80	CACUGCACUCCAGCCUGGCU
76	brain-mir-83	CAGGGUCUCGUUCUGUUGCC
112	brain-mir-88	UCUUCACCUGCCUCUGCCUGCA

SUPPLEMENTAL TABLE 3

Significantly up- or down-regulated miRNAs in MCI vs. controls.					
SEQ ID NO	Marker	median MCI	median Control	t-test p-value single	AUC
171	hsa-miR-29c-3p	31.34210526	74.84022556	1.39E−07	0.061363636
172	hsa-miR-29a-3p	39.20676692	74.4943609	2.00E−06	0.093181818
173	hsa-let-7e-5p	5465.075188	8212.93609	5.97E−06	0.139772727
174	hsa-let-7a-5p	9288.364662	15370.05263	1.19E−05	0.110227273
175	hsa-let-7f-5p	8601.315789	12867.95489	1.48E−05	0.1125

SUPPLEMENTAL TABLE 3-continued

Significantly up- or down-regulated miRNAs in MCI vs. controls.

SEQ ID NO	Marker	median MCI	median Control	t-test p-value single	AUC
176	hsa-miR-29b-3p	9.746240602	23.65037594	9.48E−05	0.160227273
177	hsa-miR-98	98.17293233	217.3533835	0.00019379	0.152272727
178	hsa-miR-425-5p	5634.067669	2907.421053	0.000351963	0.818181818
179	hsa-miR-223-3p	328.8571429	470.6090226	0.000468269	0.230681818
180	hsa-miR-181a-2-3p	241.5451128	173.3458647	0.000505662	0.805681818
181	hsa-miR-148b-3p	137.6541353	279.3120301	0.000811319	0.194318182
182	brain-mir-145	9.477443609	17.7556391	0.000969848	0.209090909
183	hsa-miR-548h-5p	4.864661654	10.08458647	0.000996949	0.198863636
184	hsa-miR-550a-5p	64.54323308	39.41353383	0.001127581	0.807954545
185	hsa-miR-374b-5p	10.30639098	20.54511278	0.001150103	0.222727273
186	hsa-miR-339-3p	126.4360902	87.83458647	0.00120356	0.811363636
187	hsa-miR-3661	1.357142857	3.716165414	0.001208331	0.210227273
188	brain-mir-190	6.342105263	2.359022556	0.001522223	0.818181818
189	brain-mir-188	6.342105263	2.359022556	0.001522223	0.818181818
190	brain-mir-189	6.342105263	2.359022556	0.001522223	0.818181818
191	brain-mir-192	6.342105263	2.359022556	0.001522223	0.818181818
192	hsa-miR-550a-3-5p	54.72368421	31.96804511	0.001581747	0.759090909
193	hsa-miR-199a-3p	4.171052632	12.45488722	0.001641108	0.204545455
194	hsa-miR-199b-3p	4.221804511	12.62593985	0.001650922	0.205681818
195	hsa-miR-660-5p	35.97744361	72.91165414	0.001678456	0.221590909
196	hsa-miR-190a	1.609022556	5.597744361	0.001784374	0.204545455
197	brain-mir-220	48.59022556	24.45112782	0.002184462	0.790909091
198	hsa-miR-548g-5p	3.447368421	9.629699248	0.002357652	0.225
199	hsa-miR-548ar-5p	3.447368421	9.629699248	0.002357652	0.225
200	hsa-miR-548x-5p	3.447368421	9.629699248	0.002357652	0.225
201	hsa-miR-548aj-5p	3.447368421	9.629699248	0.002357652	0.225
202	brain-mir-394	2.603383459	7.836466165	0.002559946	0.215909091
203	brain-mir-149	2.603383459	7.836466165	0.002559946	0.215909091
204	brain-mir-151	2.603383459	7.836466165	0.002559946	0.215909091
205	hsa-let-7c	6816.890977	11249.52256	0.002574232	0.196590909
206	brain-mir-333	2.603383459	7.836466165	0.002690942	0.215909091
207	brain-mir-170	2.603383459	7.836466165	0.002759117	0.225
208	hsa-miR-152	12.7443609	22.23120301	0.00331602	0.222727273
209	hsa-miR-15a-5p	632.3984962	1472.996241	0.003376847	0.2
210	hsa-miR-197-5p	0.830827068	0.135338346	0.00340422	0.811363636
211	brain-mir-399	21.7518797	12.77067669	0.003703683	0.781818182
212	hsa-miR-3158-3p	433.6691729	309.3571429	0.003815704	0.732954545
213	brain-mir-150	12.15413534	19.48120301	0.003816641	0.284090909
214	hsa-miR-424-3p	194.537594	105.6146617	0.003852425	0.775
215	hsa-miR-148a-3p	578.1203008	845.5263158	0.004120012	0.240909091
216	hsa-miR-3200-3p	16.64473684	23.5037594	0.004405877	0.303409091
217	hsa-miR-628-3p	2.796992481	7.116541353	0.004410063	0.243181818
218	hsa-let-7d-5p	412.6240602	598.4849624	0.004602573	0.217045455
219	hsa-miR-4781-3p	13.96616541	10.07142857	0.004719502	0.769318182
220	brain-mir-160	17.84210526	9.364661654	0.005169293	0.768181818
221	hsa-miR-374a-5p	1.793233083	5.186090226	0.005650498	0.276136364
222	hsa-miR-338-3p	0.593984962	2.716165414	0.006017454	0.302272727
223	hsa-miR-340-5p	8.187969925	21.98496241	0.006522277	0.252272727
224	brain-mir-395	5.890977444	4.323308271	0.006577993	0.719318182
225	brain-mir-308	5.890977444	4.323308271	0.006577993	0.719318182
226	brain-mir-53	5.757518797	3.890977444	0.006988766	0.7125
227	brain-mir-229	0.417293233	1.864661654	0.007037494	0.192045455
228	hsa-miR-151a-3p	3088.518797	1892.661654	0.00727488	0.713636364
229	hsa-miR-1234	2.323308271	5.62406015	0.00831879	0.270454545
230	hsa-miR-874	6.437969925	10.02631579	0.008872069	0.269318182
231	hsa-miR-548av-5p	3.906015038	9.654135338	0.008945083	0.245454545
232	hsa-miR-548k	3.906015038	9.654135338	0.008945083	0.245454545
233	brain-mir-101	3.883458647	6.633458647	0.009086578	0.271590909
234	hsa-miR-30d-5p	10223.82707	7038.496241	0.009299073	0.729545455
235	hsa-miR-3200-5p	22	37.82706767	0.00954828	0.282954545

SUPPLEMENTAL TABLE 4

Overview of miRNA markers, including sequence information

1	hsa-miR-144-5p	GGAUAUCAUCAUAUACUGUAAG
2	hsa-let-7f-5p	UGAGGUAGUAGAUUGUAUAGUU
3	hsa-let-7e-5p	UGAGGUAGGAGGUUGUAUAGUU
4	hsa-let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU
5	hsa-miR-107	AGCAGCAUUGUACAGGGCUAUCA
6	hsa-let-7g-5p	UGAGGUAGUAGUUUGUACAGUU
7	hsa-miR-103a-3p	AGCAGCAUUGUACAGGGCUAUGA
8	hsa-miR-98	UGAGGUAGUAAGUUGUAUUGUU
9	hsa-miR-29c-3p	UAGCACCAUUUGAAAUCGGUUA
10	hsa-miR-101-3p	UACAGUACUGUGAUAACUGAA
11	hsa-miR-548h-5p	AAAAGUAAUCGCGGUUUUUGUC
12	hsa-miR-106b-3p	CCGCACUGUGGGUACUUGCUGC
13	hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG
14	hsa-miR-548g-5p	UGCAAAAGUAAUUGCAGUUUUUG
15	hsa-miR-548ar-5p	AAAAGUAAUUGCAGUUUUUGC
16	hsa-miR-548x-5p	UGCAAAAGUAAUUGCAGUUUUUG
17	hsa-miR-548aj-5p	UGCAAAAGUAAUUGCAGUUUUUG
18	hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU
19	brain-mir-394	AAAAGUAAUCGUAGUUUUUG
20	hsa-miR-1294	UGUGAGGUUGGCAUUGUUGUCU
21	brain-mir-170	AAAAGUAAUGGCAGUUUUUG
22	hsa-miR-199a-3p	ACAGUAGUCUGCACAUUGGUUA
23	brain-mir-149	AAAAGUAAUCGCACUUUUUG
24	brain-mir-151	AAAAGUAAUCGCACUUUUUG
25	brain-mir-370	GGCUGGUCUGAUGGUAGUGGGUUA
26	hsa-miR-199b-3p	ACAGUAGUCUGCACAUUGGUUA
27	brain-mir-333	AAAAGUAAUCGCAGGUUUUG
28	hsa-miR-628-3p	UCUAGUAAGAGUGGCAGUCGA
29	hsa-miR-190a	UGAUAUGUUUGAUAUAUUAGGU
30	hsa-miR-29b-3p	UAGCACCAUUUGAAAUCAGUGUU
31	hsa-miR-660-5p	UACCCAUUGCAUAUCGGAGUUG
32	hsa-miR-143-3p	UGAGAUGAAGCACUGUAGCUC
33	hsa-miR-548av-5p	AAAAGUACUUGCGGAUUU
34	hsa-miR-548k	AAAAGUACUUGCGGAUUUUGCU
35	hsa-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA
36	hsa-miR-548i	AAAAGUAAUUGCGGAUUUUGCC
37	hsa-miR-17-3p	ACUGCAGUGAAGGCACUUGUAG
38	brain-mir-398	GGCUGGUCCGAGUGCAGUGGUGUU
39	hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU

SUPPLEMENTAL TABLE 4-continued

Overview of miRNA markers, including sequence information

40	hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG
41	brain-mir-150	UGAGGUAGUAGGUGGUGUGC
42	hsa-let-7i-5p	UGAGGUAGUAGUUUGUGCUGUU
43	hsa-miR-33b-5p	GUGCAUUGCUGUUGCAUUGC
44	hsa-miR-3200-3p	CACCUUGCGCUACUCAGGUCUG
45	hsa-miR-548o-5p	AAAAGUAAUUGCGGUUUUUGCC
46	hsa-miR-152	UCAGUGCAUGACAGAACUUGG
47	hsa-miR-548am-5p	AAAAGUAAUUGCGGUUUUUGCC
48	hsa-miR-548au-5p	AAAAGUAAUUGCGGUUUUUGC
49	hsa-miR-548c-5p	AAAAGUAAUUGCGGUUUUUGCC
50	brain-mir-248S	GGCGGCGGAGGCGGCGGUG
51	hsa-miR-215	AUGACCUAUGAAUUGACAGAC
52	hsa-miR-340-5p	UUAUAAAGCAAUGAGACUGAUU
53	hsa-miR-1301	UUGCAGCUGCCUGGGAGUGACUUC
54	brain-mir-145	AAGCACUGCCUUUGAACCUGA
55	hsa-miR-504	AGACCCUGGUCUGCACUCUAUC
56	hsa-miR-30d-5p	UGUAAACAUCCCCGACUGGAAG
57	hsa-miR-4781-3p	AAUGUUGGAAUCCUCGCUAGAG
58	hsa-miR-151a-3p	CUAGACUGAAGCUCCUUGAGG
59	brain-mir-112	AGCUCUGUCUGUGUCUCUAGG
60	hsa-miR-28-3p	CACUAGAUUGUGAGCUCCUGGA
61	hsa-miR-26b-3p	CCUGUUCUCCAUUACUUGGCUC
62	hsa-miR-1468	CUCCGUUUGCCUGUUUCGCUG
63	hsa-miR-128	UCACAGUGAACCGGUCUCUUU
64	hsa-miR-550a-5p	AGUGCCUGAGGGAGUAAGAGCCC
65	hsa-miR-5010-3p	UUUUGUGUCUCCCAUUCCCCAG
66	hsa-miR-148b-5p	AAGUUCUGUUAUACACUCAGGC
67	brain-mir-395	CACUGCACUCCAGCCUGGGUGA
68	brain-mir-308	CACUGCACUCCAGCCUGGGUGA
69	hsa-miR-1285-5p	GAUCUCACUUUGUUGCCCAGG
70	hsa-miR-5001-3p	UUCUGCCUCUGUCCAGGUCCUU
71	hsa-miR-3127-3p	UCCCCUUCUGCAGGCCUGCUGG
72	hsa-miR-3157-3p	CUGCCCUAGUCUAGCUGAAGCU
73	brain-mir-431	CUCGGCCUUUGCUCGCAGCACU
74	hsa-miR-550a-3-5p	AGUGCCUGAGGGAGUAAGAG
75	hsa-miR-361-5p	UUAUCAGAAUCUCCAGGGGUAC
76	brain-mir-83	CAGGGUCUCGUUCUGUUGCC
77	hsa-miR-589-5p	UGAGAACCACGUCUGCUCUGAG

SUPPLEMENTAL TABLE 4-continued

Overview of miRNA markers, including sequence information

78	hsa-miR-425-5p	AAUGACACGAUCACUCCCGUUGA
79	hsa-miR-30a-5p	UGUAAACAUCCUCGACUGGAAG
80	brain-mir-79	CACUGCACUCCAGCCUGGCU
81	brain-mir-80	CACUGCACUCCAGCCUGGCU
82	hsa-miR-330-5p	UCUCUGGGCCUGUGUCUUAGGC
83	hsa-miR-186-5p	CAAAGAAUUCUCCUUUUGGGCU
84	brain-mir-390	ACUGCAACCUCCACCUCCUGGGU
85	hsa-let-7d-3p	CUAUACGACCUGCUGCCUUUCU
86	hsa-miR-328	CUGGCCCUCUCUGCCCUUCCGU
87	hsa-miR-30c-5p	UGUAAACAUCCUACACUCUCAGC
88	brain-mir-200	UUCCUGGCUCUCUGUUGCACA
89	hsa-miR-363-3p	AAUUGCACGGUAUCCAUCUGUA
90	hsa-miR-339-3p	UGAGCGCCUCGACGACAGAGCCG
91	brain-mir-114	CACUGCAACCUCUGCCUCCGGU
92	hsa-miR-942	UCUUCUCUGUUUUGGCCAUGUG
93	hsa-miR-345-5p	GCUGACUCCUAGUCCAGGGCUC
94	brain-mir-247	ACGCCCACUGCUUCACUUGACUAG
95	hsa-miR-4742-3p	UCUGUAUUCUCCUUUGCCUGCAG
96	brain-mir-314	ACUCCCACUGCUUCACUUGAUUAG
97	brain-mir-12	ACUCCCACUGCUUGACUUGACUAG
98	brain-mir-232	UUGCUCUGCUCUCCCUUGUACU
99	brain-mir-424S	CACUGCACUCCAGCCUGGGUA
100	brain-mir-219	UCAAGUGUCAUCUGUCCCUAGG
101	hsa-miR-10a-5p	UACCCUGUAGAUCCGAAUUUGUG
102	hsa-miR-3605-3p	CCUCCGUGUUACCUGUCCUCUAG
103	brain-mir-52	CUGCACUCCAGCCUGGGCGAC
104	brain-mir-53	CCCAGGACAGUUUCAGUGAUG
105	hsa-miR-3157-5p	UUCAGCCAGGCUAGUGCAGUCU
106	brain-mir-41S	CCCCGCGCAGGUUCGAAUCCUG
107	brain-mir-201	CACCCCACCAGUGCAGGCUG
108	hsa-miR-5006-3p	UUUCCCUUUCCAUCCUGGCAG
109	hsa-miR-4659a-3p	UUUCUUCUUAGACAUGGCAACG
110	brain-mir-279	AUCCCACCGCUGCCACAC
111	brain-mir-111	CACUGCUAAAUUUGGCUGGCUU
112	brain-mir-88	UCUUCACCUGCCUCUGCCUGCA
113	brain-mir-251	UGGCCCAAGACCUCAGACC
114	hsa-miR-4435	AUGGCCAGAGCUCACACAGAGG
115	hsa-miR-5690	UCAGCUACUACCUCUAUUAGG
116	brain-mir-166	CUGGCUGCUUCCCUUGGUCU

SUPPLEMENTAL TABLE 4-continued

Overview of miRNA markers, including sequence information

117	brain-mir-193	AUCCCUUUAUCUGUCCUCUAGG
118	hsa-miR-625-5p	AGGGGGAAAGUUCUAUAGUCC
119	hsa-miR-10b-5p	UACCCUGUAGAACCGAAUUUGUG
120	brain-mir-299	CAUGCCACUGCACUCCAGCCU
121	brain-mir-153	CCUCUUCUCAGAACACUUCCUGG
122	hsa-miR-758	UUUGUGACCUGGUCCACUAACC
123	hsa-miR-30a-3p	CUUUCAGUCGGAUGUUUGCAGC
124	brain-mir-220	UCCGGAUCCGGCUCCGCGCCU
125	brain-mir-392	CCCGCCUGUCUCUCUCUUGCA
126	brain-mir-102	UAUGGAGGUCUCUGUCUGGCU
127	hsa-let-7b-3p	CUAUACAACCUACUGCCUUCCC
128	hsa-miR-340-3p	UCCGUCUCAGUUACUUUAUAGC
129	hsa-miR-484	UCAGGCUCAGUCCCCUCCCGAU
130	hsa-miR-30e-3p	CUUUCAGUCGGAUGUUUACAGC
131	brain-mir-72S	GACCACACUCCAUCCUGGGC
132	hsa-miR-371b-5p	ACUCAAAAGAUGGCGGCACUUU
133	hsa-miR-5581-3p	UUCCAUGCCUCCUAGAAGUUCC
134	brain-mir-399	CACUGCAACCUCUGCCUCC
135	brain-mir-403	AAAGACUUCCUUCUCUCGCCU
136	brain-mir-73	UCCGGAUGUGCUGACCCCUGCG
137	brain-mir-190	CCUGACCCCCAUGUCGCCUCUGU
138	brain-mir-188	CCUGACCCCCAUGUCGCCUCUGU
139	brain-mir-189	CCUGACCCCCAUGUCGCCUCUGU
140	brain-mir-192	CCUGACCCCCAUGUCGCCUCUGU
141	brain-mir-311	CACUGCAACCUCUGCCUCCCGA
142	brain-mir-161	CUUCGAAAGCGGCUUCGGCU
143	hsa-miR-3074-5p	GUUCCUGCUGAACUGAGCCAG
144	hsa-miR-30b-5p	UGUAAACAUCCUACACUCAGCU
145	hsa-miR-576-5p	AUUCUAAUUUCUCCACGUCUUU
146	brain-mir-23	UUAGUGGCUCCCUCUGCCUGCA
147	hsa-miR-943	CUGACUGUUGCCGUCCUCCAG
148	brain-mir-351	UGUCUUGCUCUGUUGCCCAGGU
149	hsa-miR-4772-3p	CCUGCAACUUUGCCUGAUCAGA
150	brain-mir-319	CUGCACUCCAGCCUGGGCGA
151	hsa-miR-937	AUCCGCGCUCUGACUCUCUGCC
152	hsa-miR-181a-2-3p	ACCACUGACCGUUGACUGUACC
153	hsa-miR-4755-5p	UUUCCCUUCAGAGCCUGGCUUU
154	hsa-miR-3909	UGUCCUCUAGGGCCUGCAGUCU

SUPPLEMENTAL TABLE 4-continued

Overview of miRNA markers, including sequence information

155	hsa-miR-1260b	AUCCCACCACUGCCACCAU
156	brain-mir-293	UUGGUGAGGACCCCAAGCUCGG
157	brain-mir-160	CACUGCAACCUCUGCCUCC
158	hsa-miR-2110	UUGGGGAAACGGCCGCUGAGUG
159	hsa-miR-584-3p	UCAGUUCCAGGCCAACCAGGCU
160	brain-mir-129	CAUGGUCCAUUUUGCUCUGCU
161	hsa-miR-1280	UCCCACCGCUGCCACCC
162	hsa-miR-3180-5p	CUUCCAGACGCUCCGCCCCACGUCG
163	hsa-miR-668	UGUCACUCGGCUCGGCCCACUAC
164	hsa-miR-4512	CAGGGCCUCACUGUAUCGCCCA
165	hsa-miR-641	AAAGACAUAGGAUAGAGUCACCUC
166	hsa-miR-1233	UGAGCCCUGUCCUCCCGCAG
167	hsa-miR-378a-5p	CUCCUGACUCCAGGUCCUGUGU
168	hsa-miR-26a-5p	UUCAAGUAAUCCAGGAUAGGCU
169	brain-mir-258	AUCCCACCCCUGCCCCCA
170	hsa-miR-1260a	AUCCCACCUCUGCCACCA
171	hsa-miR-29c-3p	UAGCACCAUUUGAAAUCGGUUA
172	hsa-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA
173	hsa-let-7e-5p	UGAGGUAGGAGGUUGUAUAGUU
174	hsa-let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU
175	hsa-let-7f-5p	UGAGGUAGUAGAUUGUAUAGUU
176	hsa-miR-29b-3p	UAGCACCAUUUGAAAUCAGUGUU
177	hsa-miR-98	UGAGGUAGUAAGUUGUAUUGUU
178	hsa-miR-425-5p	AAUGACACGAUCACUCCCGUUGA
179	hsa-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA
180	hsa-miR-181a-2-3p	ACCACUGACCGUUGACUGUACC
181	hsa-miR-148b-3p	UCAGUGCAUCACAGAACUUUGU
182	brain-mir-145	AAGCACUGCCUUUGAACCUGA
183	hsa-miR-548h-5p	AAAAGUAAUCGCGGUUUUUGUC
184	hsa-miR-550a-5p	AGUGCCUGAGGGAGUAAGAGCCC
185	hsa-miR-374b-5p	AUAUAAUACAACCUGCUAAGUG
186	hsa-miR-339-3p	UGAGCGCCUCGACGACAGAGCCG
187	hsa-miR-3661	UGACCUGGGACUCGGACAGCUG
188	brain-mir-190	CCUGACCCCCAUGUCGCCUCUGU
189	brain-mir-188	CCUGACCCCCAUGUCGCCUCUGU
190	brain-mir-189	CCUGACCCCCAUGUCGCCUCUGU
191	brain-mir-192	CCUGACCCCCAUGUCGCCUCUGU
192	hsa-miR-550a-3-5p	AGUGCCUGAGGGAGUAAGAG
193	hsa-miR-199a-3p	ACAGUAGUCUGCACAUUGGUUA

SUPPLEMENTAL TABLE 4-continued

Overview of miRNA markers, including sequence information

194	hsa-miR-199b-3p	ACAGUAGUCUGCACAUUGGUUA
195	hsa-miR-660-5p	UACCCAUUGCAUAUCGGAGUUG
196	hsa-miR-190a	UGAUAUGUUUGAUAUAUUAGGU
197	brain-mir-220	UCCGGAUCCGGCUCCGCGCCU
198	hsa-miR-548g-5p	UGCAAAAGUAAUUGCAGUUUUUG
199	hsa-miR-548ar-5p	AAAAGUAAUUGCAGUUUUUGC
200	hsa-miR-548x-5p	UGCAAAAGUAAUUGCAGUUUUUG
201	hsa-miR-548aj-5p	UGCAAAAGUAAUUGCAGUUUUUG
202	brain-mir-394	AAAAGUAAUCGUAGUUUUUG
203	brain-mir-149	AAAAGUAAUCGCACUUUUUG
204	brain-mir-151	AAAAGUAAUCGCACUUUUUG
205	hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU
206	brain-mir-333	AAAAGUAAUCGCAGGUUUUG
207	brain-mir-170	AAAAGUAAUGGCAGUUUUUG
208	hsa-miR-152	UCAGUGCAUGACAGAACUUGG
209	hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG
210	hsa-miR-197-5p	CGGGUAGAGAGGGCAGUGGGAGG
211	brain-mir-399	CACUGCAACCUCUGCCUCC
212	hsa-miR-3158-3p	AAGGGCUUCCUCUCUGCAGGAC
213	brain-mir-150	UGAGGUAGUAGGUGGUGUGC
214	hsa-miR-424-3p	CAAAACGUGAGGCGCUGCUAU
215	hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU
216	hsa-miR-3200-3p	CACCUUGCGCUACUCAGGUCUG
217	hsa-miR-628-3p	UCUAGUAAGAGUGGCAGUCGA
218	hsa-let-7d-5p	AGAGGUAGUAGGUUGCAUAGUU
219	hsa-miR-4781-3p	AAUGUUGGAAUCCUCGCUAGAG
220	brain-mir-160	CACUGCAACCUCUGCCUCC
221	hsa-miR-374a-5p	UUAUAAUACAACCUGAUAAGUG
222	hsa-miR-338-3p	UCCAGCAUCAGUGAUUUUGUUG
223	hsa-miR-340-5p	UUAUAAAGCAAUGAGACUGAUU
224	brain-mir-395	CACUGCACUCCAGCCUGGGUGA
225	brain-mir-308	CACUGCACUCCAGCCUGGGUGA
226	brain-mir-53	CCCAGGACAGUUUCAGUGAUG
227	brain-mir-229	AUCCCACCUCUGCUACCA
228	hsa-miR-151a-3p	CUAGACUGAAGCUCCUUGAGG
229	hsa-miR-1234	UCGGCCUGACCACCCACCCCAC
230	hsa-miR-874	CUGCCCUGGCCCGAGGGACCGA
231	hsa-miR-548av-5p	AAAAGUACUUGCGGAUUU

SUPPLEMENTAL TABLE 4-continued

Overview of miRNA markers, including sequence information		
232	hsa-miR-548k	AAAAGUACUUGCGGAUUUUGCU
233	brain-mir-101	AGACCUACUUAUCUACCAACA
234	hsa-miR-30d-5p	UGUAAACAUCCCCGACUGGAAG
235	hsa-miR-3200-5p	AAUCUGAGAAGGCGCACAAGGU

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ggcugguccg agugcagugg uguu 24

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ucagugcacu acagaacuuu gu 22

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ucguaccgug aguaauaaug cg 22

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ugagguagua gguggugugc 20

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ugagguagua guuugugcug uu 22

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gugcauugcu guugcauugc 20

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aaaaguaauu gcgguuuuug cc 22

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aaaaguaauu gcgguuuuug cc 22

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uuauaaagca augagacuga uu 22

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gaucucacuu uguugcccag g 21

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cugcccuagu cuagcugaag cu 22

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cucggccuuu gcucgcagca cu 22

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agugccugag ggaguaagag 20

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uuaucagaau cuccaggggu ac 22

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cagggucucg uucuguugcc 20

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ugagaaccac gucugcucug ag 22

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aaugacacga ucacuccccgu uga 23

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uguaaacauc cucgacugga ag 22

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cacugcacuc cagccuggcu 20

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cacugcacuc cagccuggcu 20

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ucucugggcc ugugucuuag gc 22

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caaagaauuc uccuuuuggg cu 22

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acugcaaccu ccaccuccug ggu 23

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cuauacgacc ugcugccuuu cu 22

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cuggcccucu cugcccuucc gu 22

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uguaaacauc cuacacucuc agc 23

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uuccuggcuc ucuguugcac a 21

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aauugcacgg uauccaucug ua 22

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ugagcgccuc gacgacagag ccg 23

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cacugcaacc ucugccuccg gu 22

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<400> SEQUENCE: 92

ucuucucugu uuuggccaug ug 22

<210> SEQ ID NO 93
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gcugacuccu aguccagggc uc 22

<210> SEQ ID NO 94
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<400> SEQUENCE: 94

acgcccacug cuucacuuga cuag 24

<210> SEQ ID NO 95
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<400> SEQUENCE: 95

ucuguauucu ccuuugccug cag 23

<210> SEQ ID NO 96
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<400> SEQUENCE: 96

acucccacug cuucacuuga uuag 24

<210> SEQ ID NO 97
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<400> SEQUENCE: 97

acucccacug cuugacuuga cuag 24

<210> SEQ ID NO 98
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<400> SEQUENCE: 98

uugcucugcu cucccuugua cu 22

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cacugcacuc cagccugggu a 21

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<400> SEQUENCE: 100

ucaaguguca ucugucccua gg 22

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uacccuguag auccgaauuu gug 23

<210> SEQ ID NO 102
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ccuccguguu accuguccuc uag 23

<210> SEQ ID NO 103
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<400> SEQUENCE: 103

cugcacucca gccugggcga c 21

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<400> SEQUENCE: 104

cccaggacag uuucagugau g 21

<210> SEQ ID NO 105
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<400> SEQUENCE: 105

uucagccagg cuagugcagu cu 22

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<400> SEQUENCE: 106

ccccgcgcag guucgaaucc ug 22

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<400> SEQUENCE: 107

caccccacca gugcaggcug 20

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<400> SEQUENCE: 108

uuucccuuuc cauccuggca g 21

<210> SEQ ID NO 109
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uuucuucuua gacauggcaa cg 22

<210> SEQ ID NO 110

<211> LENGTH: 18
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aucccaccgc ugccacac 18

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cacugcuaaa uuuggcuggc uu 22

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ucuucaccug ccucugccug ca 22

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<400> SEQUENCE: 113

uggcccaaga ccucagacc 19

<210> SEQ ID NO 114
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<212> TYPE: RNA
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<400> SEQUENCE: 114

auggccagag cucacacaga gg 22

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ucagcuacua ccucuauuag g 21

<210> SEQ ID NO 116
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cuggcugcuu cccuuggucu 20

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aucccuuuau cuguccucua gg 22

<210> SEQ ID NO 118
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agggggaaag uucuauaguc c 21

<210> SEQ ID NO 119
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<400> SEQUENCE: 119

uacccuguag aaccgaauuu gug 23

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caugccacug cacuccagcc u 21

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ccucuucuca gaacacuucc ugg 23

<210> SEQ ID NO 122
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uuugugaccu gguccacuaa cc 22

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<400> SEQUENCE: 123

cuuucagucg gauguuugca gc 22

<210> SEQ ID NO 124
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<400> SEQUENCE: 124

uccggauccg gcuccgcgcc u 21

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<400> SEQUENCE: 125

cccgccuguc ucucucuugc a 21

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<400> SEQUENCE: 126

uauggagguc ucugucuggc u 21

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<400> SEQUENCE: 127

cuauacaacc uacugccuuc cc 22

<210> SEQ ID NO 128
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<400> SEQUENCE: 128

uccgucucag uuacuuuaua gc 22

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<400> SEQUENCE: 129

ucaggcucag uccccucccg au 22

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cuuucagucg gauguuuaca gc 22

<210> SEQ ID NO 131
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<400> SEQUENCE: 131

gaccacacuc cauccugggc 20

<210> SEQ ID NO 132
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<400> SEQUENCE: 132

acucaaaaga uggcggcacu uu 22

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uuccaugccu ccuagaaguu cc 22

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<400> SEQUENCE: 134

cacugcaacc ucugccucc 19

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<400> SEQUENCE: 135

aaagacuucc uucucucgcc u 21

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<400> SEQUENCE: 136

uccggaugug cugaccccug cg 22

<210> SEQ ID NO 137
<211> LENGTH: 23
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<400> SEQUENCE: 137

ccugaccccc augucgccuc ugu 23

<210> SEQ ID NO 138
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<400> SEQUENCE: 138

ccugaccccc augucgccuc ugu 23

<210> SEQ ID NO 139
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<400> SEQUENCE: 139

ccugaccccc augucgccuc ugu 23

<210> SEQ ID NO 140
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<400> SEQUENCE: 140

ccugaccccc augucgccuc ugu 23

<210> SEQ ID NO 141
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 141

cacugcaacc ucugccuccc ga 22

<210> SEQ ID NO 142
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 142

cuucgaaagc ggcuucggcu 20

<210> SEQ ID NO 143
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<400> SEQUENCE: 143

guuccugcug aacugagcca g 21

<210> SEQ ID NO 144
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<400> SEQUENCE: 144

uguaaacauc cuacacucag cu 22

<210> SEQ ID NO 145
<211> LENGTH: 22
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<400> SEQUENCE: 145

auucuaauuu cuccacgucu uu 22

<210> SEQ ID NO 146
<211> LENGTH: 22
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<400> SEQUENCE: 146

uuaguggcuc ccucugccug ca 22

<210> SEQ ID NO 147
<211> LENGTH: 21
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<400> SEQUENCE: 147

cugacuguug ccguccucca g 21

<210> SEQ ID NO 148
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<400> SEQUENCE: 148

ugucuugcuc uguugcccag gu 22

<210> SEQ ID NO 149
<211> LENGTH: 22
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<400> SEQUENCE: 149

ccugcaacuu ugccugauca ga 22

<210> SEQ ID NO 150
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cugcacucca gccugggcga 20

<210> SEQ ID NO 151
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<400> SEQUENCE: 151

auccgcgcuc ugacucucug cc 22

<210> SEQ ID NO 152
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<400> SEQUENCE: 152

accacugacc guugacugua cc 22

<210> SEQ ID NO 153
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<400> SEQUENCE: 153

uuucccuuca gagccuggcu uu 22

<210> SEQ ID NO 154
<211> LENGTH: 22
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<400> SEQUENCE: 154

uguccucuag ggccugcagu cu 22

<210> SEQ ID NO 155
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<400> SEQUENCE: 155

aucccaccac ugccaccau 19

<210> SEQ ID NO 156
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<400> SEQUENCE: 156

uuggugagga ccccaagcuc gg 22

<210> SEQ ID NO 157
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<400> SEQUENCE: 157

cacugcaacc ucugccucc 19

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uuggggaaac ggccgcugag ug 22

<210> SEQ ID NO 159
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ucaguuccag gccaaccagg cu 22

<210> SEQ ID NO 160
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What is claimed is:

1. A method of treating Alzheimer's Disease in a patient in need thereof, said method comprising

administering an anti-Alzheimer's Disease therapy to the patient, wherein a blood sample from the patient exhibits an expression level value of at least one miRNA selected from the group consisting of SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 7, SEQ ID NO 56, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 64, SEQ ID NO 65, SEQ ID NO 66, SEQ

ID NO 69, SEQ ID NO 70, SEQ ID NO 71, SEQ ID NO 72, SEQ ID NO 73, SEQ ID NO 78, SEQ ID NO 85, SEQ ID NO 142 and SEQ ID NO 236 compared to a reference expression level value.

2. The method according to claim **1**, wherein the at least one miRNA from the sample is selected from the group consisting of SEQ ID NO 59, SEQ ID NO 65, SEQ ID NO 1, and SEQ ID NO 56.

3. The method according to claim **1**, wherein the at least one miRNA from the sample includes all seven of brain-miR-112, hsa-miR-5010-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-let-7d-3p, hsa-miR-532-5p, and brain-miR-161.

4. The method according to claim **1**, wherein the at least one miRNA from the sample includes a set of 5 miRNAs selected from the group consisting of

brain-mir-112 hsa-miR-5010-3p hsa-miR-1285-5p hsa-miR-151a-3p hsa-let-7f-5p,
hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-3157-3p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-1285-5p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7a-5p,
hsa-miR-5001-3p hsa-miR-1285-5p hsa-miR-425-5p hsa-miR-148b-5p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-1285-5p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,
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hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-miR-144-5p hsa-let-7a-5p,
hsa-miR-1285-5p brain-mir-112 hsa-miR-425-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-5001-3p hsa-miR-1285-5p brain-mir-112 hsa-miR-151a-3p hsa-let-7f-5p,
hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-148b-5p hsa-miR-144-5p hsa-let-7f-5p,
hsa-miR-1285-5p hsa-miR-3157-3p hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-5001-3p hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p,
brain-mir-431 hsa-miR-1285-5p hsa-miR-3157-3p hsa-miR-151a-3p hsa-miR-144-5p,
hsa-miR-3127-3p hsa-miR-1285-5p brain-mir-112 hsa-miR-425-5p hsa-miR-151a-3p,
hsa-miR-1285-5p hsa-miR-5010-3p hsa-miR-151a-3p hsa-miR-144-5p hsa-let-7f-5p,
hsa-miR-550a- 5phsa-miR-1285-5p brain-mir-112 hsa-miR-151a-3p hsa-let-7f-5p,
hsa-miR-1285-5p brain-mir-112 hsa-miR-148b-5p hsa-miR-151a-3p hsa-miR-144-5p, and
hsa-miR-5001-3p brain-mir-112 hsa-miR-5010-3p hsa-miR-151a-3p hsa-let-7f-5p.

5. The method according to claim **4**, further comprising adding the expression level values of the set of five miRNAs.

6. The method according to claim **1**, wherein the determination of the expression level value of the at least one miRNA from the sample is obtained by use of a method selected from the group consisting of a sequencing-based method, an array-based method and a PCR-based method.

7. The method according to claim **1**, wherein the expression levels values of at least 2 miRNAs in the sample are determined.

8. The method according to claim **1**, wherein the blood sample from the patient also exhibits an expression level value of at least one miRNA selected from the group consisting of hsa-miR-26b-3p, hsa-miR-26a-5p, brain-miR-161 and hsa-miR-5010-3p compared to a reference expression level value.

9. A kit for performing the method according to claim **1**, said kit comprising

means for determining in said blood sample from said patient, an expression level value of at least one miRNA selected from the group consisting of miRNAs having the sequence SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 7, SEQ ID NO 56, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 64, SEQ ID NO 65, SEQ ID NO 66, SEQ ID NO 69, SEQ ID NO 70, SEQ ID NO 71, SEQ ID NO 72, SEQ ID NO 73, SEQ ID NO 78, SEQ ID NO 85, SEQ ID NO 142 and SEQ ID NO 236.

10. The kit of claim **9**, further comprising at least one reference pattern of expression levels values for comparing with the expression level values of the at least one miRNA from said sample.

11. The method according to claim **7**, wherein the expression level values of at least 3 miRNAs in the sample are determined.

12. The method according to claim **11**, wherein the expression level values of at least 4 miRNAs in the sample are determined.

13. The method according to claim **12**, wherein the expression level values of at least 5 miRNAs in the sample are determined.

14. The method according to claim **13**, wherein the expression level values of at least 6 miRNAs in the sample are determined.

15. The method according to claim **14**, wherein the expression level values of at least 7 miRNAs in the sample are determined.

16. The method according to claim **15**, wherein the expression level values of at least 8 miRNAs in the sample are determined.

17. The method according to claim **16**, wherein the expression level values of at least 9 miRNAs in the sample are determined.

18. The method according to claim **17**, wherein the expression level values of at least 10 miRNAs in the sample are determined.

19. The method according to claim **18**, wherein the expression level values of at least 12 miRNAs in the sample are determined.

* * * * *