Biomarker identification in neurologic diseases: improving diagnostics and therapeutics

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Identification of biomarkers in neurological disease remains impeded by many obstacles. Among them are the availability of tissue at the site of pathology, poor clinical diagnostics, the complexity of the brain and a general dearth of functional end points and models for validation. However, advances in technology have helped to overcome these challenges. Some of these advances include standardization and increased efficiency in brain banking, novel techniques for brain imaging, improved methods for reducing tissue heterogeneity including laser capture microdissection, high-throughput genomics, new functional validation techniques such as RNA interference, and the development of new animal models of neurologic disease. In order to efficiently handle the wealth of information that will be gleaned from these new technologies, new integrated databasing protocols will be necessary. Access to these databases by researchers and clinicians is critical to the continued progress being made in biomarker identification in neurological disease. These challenges and ways to overcome them are presented here in the context of a disease known to be a robust model for biomarker identification, Alzheimer's disease.

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Biomarkers have been defined by the National Institutes of Health Biomarkers Definitions Working Group as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [1]. In other words, a biomarker (or endophenotype) is any neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, neuropsychological, genetic, genomic and/or proteomic marker that is indicative of the presence of disease. There are several characteristics that should be common to biomarkers in order for them to be useful in disease predictability:

- Associated with illness in a population
- Heritable
- State independent and exist whether the disease is active or not
- Cosegregate within families
- When present in affected individuals should be found in related individuals at a higher rate than in the general population [2,3]

Although effective biomarkers should display most, if not all, of these factors, a full discussion of each of the points in relation to an individual biomarker is beyond the scope of this review. Traditionally, these biomarkers have been especially difficult to discern in neurological disease due to several factors inherent in the study of human behavior and brain pathology.

This review seeks to investigate the challenges that can be expected when attempting to identify biomarkers in neurological disease and how advancements in science have helped to overcome these challenges. The four basic challenges to biomarker identification in neurological disease that will be discussed are:

- Availability of tissue at the site of pathology
- Poor clinical diagnostics and extent of disease progression at the time of diagnosis
- Complexity of brain and tissue heterogeneity
- Lack of functional end points and models for validation

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The continued identification of these biomarkers will permit further investigations, including genetic or chemical manipulation of animal models, which can help characterize disease pathology and lead to the development of novel methods of diagnostics and/or therapeutics. As Alzheimer's disease (AD) has become a robust model for biomarker identification in neurological disease in recent years, this review will focus mainly on AD as an example. However, other examples of neurodegenerative and neurobehavioral disease will be included when appropriate.

Availability of tissue at site of pathology

Identification of biomarkers in neurological diseases is considerably more challenging than in other diseases due to a variety of factors. Many of these difficulties are related to the acquisition and quality of the necessary tissues, especially those at the actual site of pathology. Obviously, tissue availability in the affected area (i.e., brain), although potentially obtainable via the rarely performed brain biopsy, is realistically only available post-mortem [4]. By this stage, the affected tissues have likely been ravaged by the disease leaving little experimental material of good quality to investigate early etiologies [5,6].

Traditional methods for preserving post-mortem brain tissue consisted of formalin fixation, however, it has since been demonstrated that formalin fixation is a barrier to performing many modern molecular biological techniques. Thus, at present most brain banks use a variety of freezing techniques [7,8], in addition to fixation, to preserve the valuable specimens. As some biomarkers have extremely short half-lives [9], post-mortem interval (PMI), the amount of time that elapses between death and preservation, as well as the type of preservation, has become increasingly important. PMI is known to vary dramatically in brain banks across the nation from 1.6 to 32.5 h and even longer [10,11]. In addition to the dilemmas facing post-mortem neuropathological characterization of those suffering from neurologic disease, ante-mortem clinical diagnostic procedures are also difficult.

The problem of tissue availability and acquisition has been largely overcome with advances in brain banking. As has been mentioned, new freezing techniques and shorter PMIs are making higher quality tissue available more rapidly. A brain bank at Duke University has developed a rapid brain autopsy protocol, which processes brains within 1 h after death [12], and the Sun Health Research Institute in Arizona maintains an average PMI of 2.6 h [BEACH T, PERS. COMMUN.]. Institutes all over the country are attempting to achieve PMIs like this in order to standardize protocols as well as provide the highest quality samples to researchers. The expedited processing of the brain samples also minimizes the loss of tissue, thus increasing the availability of tissue at the site of pathology.

In addition to the speed at which the specimens are being processed, new computerized databasing technologies are cataloging and organizing the donor submissions in ways that maximize the amount of information available to the researcher. Detailed knowledge regarding the neuropsychiatric, neurologic, neuropathologic, and other clinical parameters of the tissue donors are now easily and rapidly available to the researcher [13]. Collectively, these general physiologic variables can also influence brain biochemistry and impact evaluation of inclusion or exclusion criteria of appropriate samples for a clinical investigation. With the new databases, it is not unusual for up to 1000 different ante- and post-mortem data factors to be generated and cataloged for a given individual. This availability of information will allow the researcher to make informed, appropriate choices about sample inclusion that will result in more informative investigations. Of course, the accuracy of this database information, particularly in relation to neurological and neuropsychiatric evaluation, is only as good as the initial clinical diagnosis.

Poor clinical diagnostics & extent of disease progression at time of diagnosis

Clinical diagnostics and (sub)stratification of patient populations are poorly developed for most neurodegenerative diseases, most notably multiple sclerosis (MS) [14,15] but also to a lesser degree with atypical forms of Parkinson's disease (PD) [16] and AD [17]. Even in the better case scenario of AD, clinicopathological diagnosis has been demonstrated to have a specificity between 76 and 88% and sensitivity between 53 and 65% [18–20], with a confirmation rate of probable AD as low as 65% [21]. Using the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) criteria, AD could only be reliably distinguished from frontotemporal dementia 23% of the time [22]. In neurodegenerative disorders as a whole, clinical diagnosis is only accurate in approximately 70–80% of cases [23].

With neurobehavioral diseases, for example schizophrenia, the problem becomes even more difficult as many symptoms, such as the presence of hallucinations and delusions, are disclosed through verbal self-report of affected individuals and often questionable second-hand patient histories from family members and/or friends [24,25]. Additionally, many of the psychotic symptoms that are the hallmarks of schizophrenia can result from other behavioral [26] and nonbehavioral insults, such as mental retardation [27] and/or brain tumors [28]. Further compounding the dilemma is the fact that the diagnoses of neurobehavioral disorders are frequently based on negative symptoms, flattened affect, refusal to speak, loss of motivation in schizophrenia [29] and lack of manic episodes in bipolar disorder [30]. These can be difficult to confirm by the clinician in outpatient scenarios and require more intensive observation, for example, hospitalization or institutionalization.

New developments in brain imaging techniques have helped overcome some of the problems associated with clinical diagnosis of neurological disorders. Used in the past primarily as a technique to exclude structural lesions as etiological in neurodegenerative disease [31], neuroimaging has become increasingly utilized as a tool for diagnosis of the conditions themselves. The American Academy of Neurology suggests that neuroimaging should be used to facilitate the diagnosis of all cases of dementia [32].

Imaging of progressive cerebral atrophy by computerized tomography (CT) has contributed to the diagnosis of dementia

since the early 1980s [33], however, the emergence of targeted therapeutics has made early diagnosis and scrutiny of disease evolution increasingly important [34,35]. Magnetic resonance imaging (MRI) extended the ability of researchers to assess the microscopic changes that accompany progressive brain atrophy. However, several drawbacks inherently associated with the cross-sectional nature of this analysis (inter individual variability, laborious manual outlining and subjectivity in defining structural boundaries) has limited the precision, and thus efficacy, of the procedure [36].

In the detection and tracking of AD, it has been helpful to characterize and measure the volume of a hippocampal region of interest [37,38]. Whereas this and other regional or whole-brain measurements can be generated separately from each image, researchers have begun to obtain some of these changes from the direct comparison of sequential, coregistered MRIs. Techniques such as the brain boundary shift integral [39,40] or an iterative principal component analysis [41] are used to compute rates of whole-brain atrophy from sequential MRIs, whereby the individual serves as his or her own control. Accordingly, researchers have demonstrated the ability to distinguish abnormally high rates of whole-brain atrophy in patients with probable AD from normal aging, with no overlap between groups, to estimate the power of this approach to assess the effects of a putative disease-modifying treatment in randomized, placebo-controlled trials. Although the findings are more preliminary, researchers have begun to use automated voxel-based image-analysis strategies to detect and track changes in regional brain size and shape [39,41] and gray matter density [43,44]. Some of these methods capitalize on the transformation of an individual's brain image, before or after it has been segmented into gray matter, white matter and cerebrospinal fluid (CSF) or different regions of interest. In the case of voxel-based morphometry (VBM), for instance, spatially standardized gray matter images can be used to detect or track alterations in gray matter in patients with probable AD.

Using a variety of visual and linear measures (e.g., qualitative visual rating scale, height of left hippocampus, radial width of temporal horn of lateral ventricle) to assess medial temporal lobe atrophy in neuroimages obtained using MRI from a number of investigations, Scheltens and coworkers calculated a corrected sensitivity and specificity for detection of AD, compared with controls, of 85 and 88%, respectively [45]. These imaging techniques are even more important in other neurodegenerative diseases (e.g., frontotemporal dementia and semantic aphasia) where asymmetric atrophy is maintained throughout the progression of the disease and helps differentiate these conditions from AD [46,47]. This has been determined as essential for the diagnosis of vascular dementia, according to the National Institute for Neurological Disorders and Stroke–Association pour la Recherche et L'Enseignement en Neurosciences, an international consortium on vascular dementia [48].

Positron emission tomography (PET) scanning, which has been used extensively to assess metabolic activity within the brains of those suffering from neurologic disease, and single-photon emission computed tomography (SPECT), which measures blood flow, can also help illustrate neuronal degeneration and/or cognitive deterioration. Imaging data from PET scans have been shown to discriminate (with 93% accuracy) mild-to-moderate AD cases from normal controls [49]. PET evaluation of relative cerebral glucose metabolic rate demonstrated that 36% of mild cognitive impairment (MCI) patients converted to AD within 1 year and thus concluded that a reduction of cerebral glucose metabolic rate in prefrontal cortical areas is associated with the transition from MCI to AD [50]. Another common pattern that has been observed in AD patients, this time using SPECT, is the progressive decrease of temporoparietal blood flow that increases with disease severity [51,52].

Neuroimaging has also become important for diagnosis [53–55], as well as testing the effectiveness of treatments [55–57], for behavioral disorders such as schizophrenia and bipolar illness. Early studies using CT repeatedly demonstrated a lateral ventricular enlargement in the brains of schizophrenic patients that could be used to forecast subsequent cognitive impairment [58]. This phenomenon was further characterized as the ventricle–brain ratio (VBR) and demonstrated to be heritable within families of schizophrenic patients [59–61], as well as those affected with bipolar disorder [62,63].

Profiles of accelerated gray matter loss, assessed by MRI, have been demonstrated repeatedly in schizophrenia [54,55,64,65] and lack of functional connectivity of brain regions, also assessed by MRI, has been implicated in the etiology of both schizophrenia [66] and bipolar disorder [67]. PET and SPECT have been used extensively to elucidate the mechanism of action of antipsychotic drugs in schizophrenia [68–71], as well as mood stabilizers in affective disorders [72–75]. For example, the principal pharmacological treatment for bipolar disorder, lithium, has been reported to be neuroprotective and to prevent gray matter volume loss in chronically treated neuropsychiatric patients [76,77].

Reduced neuronal activity in the dorsolateral prefrontal cortex (DLPFC), as assayed by PET/SPECT, is common in schizophrenia [78,79] and has been linked with such symptoms as lack of motivation and impaired abstract thinking [80]. On the contrary, increased neuronal activity in the DLPFC, measured via functional MRI (fMRI), has also been reported in association with certain cognitive tasks such as item recognition [81–83]. Some investigators have used the morphometric alterations in the DLPFC to distinguish between bipolar disorder and schizophrenia [84], which some believe to be different manifestations of psychosis along the same continuum [85–88].

Complexity of the brain & tissue heterogeneity

In many cases, the complexity of the brain itself presents a severe road block in the identification of utile biomarkers. In most organs, for example liver and muscle, cells are generally homogenous in their phenotypes, transcriptomes, proteomes and cellular interactions. This is not the case in the brain,

however, where transcriptomes, proteomes, morphological phenotypes and interactive connections vary widely within the neurons and glia. Diverse cellular experiences can thus be interpreted as differences that manifest at the biochemical and epigenetic level. Additionally, the complex experience and interactions of individuals must be taken into account along with that of their genes, proteins, cells and tissues. Thus, human behavior, the phenotypic output of the brain, is much more than the sum of its parts.

Another factor associated with inherent brain complexity that further confounds the identification of biomarkers in neurological disease is the heterogeneity of the representative neuropathologies. If the neuropathological/neuropsychiatric characterization of the sample is incorrect or unavailable to the researcher, the conclusions drawn from molecular biological and/or neurochemical investigations will be invalid.

The only way to definitively confirm a clinical diagnosis of AD, PD, Lewy body disease (LBD) and many other neurodegenerative diseases is still observation of their respective pathological traits within the brain upon autopsy. Even then, many of these neurodegenerative diseases are differentiated by a complex set of neuropathological features, which share a significant number of common characteristics. For example, immunohistochemical staining for the genetic PD biomarker, α-synuclein, identified numerous, varied, and as yet unidentified subtypes of Lewy body pathology along an LBD continuum [89], some showing LBD pathology alongside that of progressive supranuclear palsy [90] as well as AD [91]. These new findings have instigated a review of the Lewy body pathology in both PD [92] and dementia with Lewy bodies [93].

Cases of mixed pathology are more common than expected. Barker and coworkers reported that AD was present in 66 and 77% of LBD and vascular dementia patients, respectively [99]. In addition, the deposition of amyloid, a hallmark of AD, has also been demonstrated in many cases of PD [100]. Another hallmark of AD, tau pathology, is also common in frontotemporal dementia with parkinsonism [94], PD [95,96], dementia with Lewy bodies [97] and progressive supranuclear palsy [98]. Thus, diagnosis has become extremely stringent. In the case of AD, both cerebral β-amyloid deposition and neocortical neurofibrillary tangles are necessary for diagnosis [101]. Cases with either of the two lesions or cases with both lesions but in the incorrect brain location, are excluded from the diagnosis of AD and classified otherwise [7].

As there are few pathological markers discovered as yet for the neurobehavioral diseases, these observations apply mainly to the neurodegenerative diseases. Therefore, the presence of histopathological lesions in neurodegenerative diseases provides a distinct advantage over neurobehavioral diseases, which generally lack pathological entities that can help confirm or deny the previous clinical diagnosis.

Several approaches are available to help unravel the complexities of neuropathological tissue heterogeneity in the brain. One example is to purify individual cells of interest and one technique for doing so is laser capture microdissection (LCM). In the past, utilization of microarray strategies has required the use of large amounts of RNA (5–10 µg) and thus large tissue dissections from the brain. LCM, combined with new RNA amplification protocols, has permitted microarray analyses to be performed with as little as 10 ng of starting material and has revolutionized the study of transcriptomes from single cell populations [102]. Transparent, thermoplastic film on a standard glass histopathology slide to create strong focal adhesion allows selective procurement of the targeted cells by employing a carbon dioxide laser pulse that specifically activates the film above the cells of interest. LCM thus permits a rapid, one-step acquisition of selected human cell populations from a section of complex, heterogeneous tissue [103]. This breakthrough has not only helped reduce the level of tissue heterogeneity inherent to these types of investigations, it has enabled researchers to access tissue precisely at and around the site of pathology. In AD for example, RNA from individual neurons containing neurofibrillary tangles versus that from nearby nontangle-containing neurons have been isolated and examined via expression arrays. This results in expression profiles of tangled versus nontangled neurons in AD. This approach can be extended to a variety of neurologic diseases that show known histopathology (e.g., PD and LBD), as well as neuropsychiatric diseases with no known pathology, based on the biomarkers defined by neuroimaging studies.

Several groups have undertaken large-scale efforts to create publicly accessible atlas' of the human brain in an effort to help deal with the issue of tissue heterogeneity. One of these endeavors is GENSAT, the Gene Expression Nervous System Atlas [201]. This ambitious project seeks to create a database of CNS gene expression during development at the cellular level to facilitate investigation of cell-specific genetic and physiological phenomena in the brain. Utilizing a combination of *in situ* hybridization and bacterial artificial chromosome (BAC) vectors containing green fluorescent protein-tagged genes of interest in transgenic mice, Gong and coworkers have been able to identify several genes and gene pathways involved in neural specification (*Gscl*), axon target interactions (*Sema3b*), neuronal migration (*Lhx6* and *Pde1c*) and hundreds of other genes [104]. These results underscore the power of this approach for identifying and characterizing anatomical, genetic and physiological factors in specific cell populations.

The Allen Institute for Brain Science has initiated the Allen Brain Atlas [202], which proposes to capitalize on recent advances in computer science, bioinformatics, imaging analysis and the sequencing of the human genome. The aim is to create the most comprehensive map possible of the brain at the cellular level, illustrating the functional anatomy of the brain through a collection of gene expression maps, brain circuits and cell locations. In the future, the Allen Brain Atlas plans to extend the database to include multiple strains of mice and to compile data from nonmurine species, such as human and nonhuman primates, as well as adding pathway, connectivity, proteomics and behavioral data to augment the anatomic and genetic data.

A commercial group has also begun to produce, collect and integrate accurate, 3D volumetric data on gene expression within the mouse brain and to correlate that data with the

developing wealth of learning on the architecture and functions of brain structures, circuits and cells. Neurome, Inc., has developed several new technologies for teasing out the complexities of tissue heterogeneity in the brain, including MiceSlice™, NeuroZoom™, BrainArchive™ and BrainPrints™ [203]. These tools incorporate standardized preparation of brain section tissues followed by precise, computer-aided extraction, analyses and display of quantitative data from the resulting microscope images. These data will be archived in a publicly accessed web database (BrainArchive) that will permit integration and comparison of brain structure and circuitry data as well as automated comparison of quantitative, spatial and volumetric data from the experimental mice. These novel mechanisms will undoubtedly contribute to an increased understanding of brain structure and function at the cellular level and will likely become a model for continuing investigation into the etiology of neurological diseases.

Lack of functional end points & models for validation

Finally in the list of road blocks to obtaining neurological biomarkers is the dearth of functional and model systems for validation. Since neurological diseases are partially, or wholly, behavioral in nature, it is difficult to ascertain many of the phenotypic characteristics as they can occur *in vitro* or *in vivo*. Thus, surrogate end points (i.e., biomarkers) need to be designated, which can help to identify these characteristic pathologies without necessarily being able to observe the underlying behavioral attributes. These end points must then be functionally validated. In other words, it must be evaluated whether or not changes in surrogate end points (observed in the RNA, DNA or protein) have any measurable effect on the actual phenotype of a cell or animal model.

In general, there are two methods for *in vitro* functional validation of gene expression studies in neurological diseases. These include methods to both decrease expression of specific target genes or proteins and methods to increase expression of specific genes or proteins. These approaches can be extremely informative when combined with powerful functional validation assays that measure a specific cellular neurological phenotype.

RNA interference (RNAi) is a novel strategy to suppress gene expression and subsequently validate surrogate end points in cellular models. RNAi is an evolutionarily conserved mechanism whereby long (typically >200 nucleotides [nt]), double-stranded RNA (dsRNA) molecules specifically suppress the expression of genes bearing their complementary sequences [105]. Introduction of long dsRNA (>30 nt) in mammalian cells, however, initiates a potent antiviral response, characterized by nonspecific inhibition of protein synthesis and RNA degradation [106]. This antiviral response can be circumvented by the introduction and/or expression of small interfering RNAs (siRNAs). siRNAs are dsRNAs that have been processed into 20–25-nt RNAs by an RNase III-like enzyme, for example Dicer. The siRNAs assemble into endoribonuclease-containing complexes known as RNA-induced silencing complexes (RISCs), which become active upon linearization of the siRNAs. The unwound siRNAs serve to direct the RISCs to RNA molecules of complementary sequence, where they cleave near the middle of the region bound by the siRNA and destroy the associated RNA [107].

RNAi has been used successfully to silence gene expression in a variety of systems. Of particular relevance to the study of neurological diseases, Luo and colleagues recently used siRNA to elucidate the precise functions of several cofactors involved in presenilin (PS) 1/γ-secretase-mediated cleavage of β-amyloid precursor protein (βAPP) in AD [108]. The cofactors APH-1 and PEN-2 have been shown to physically interact with PS1 and are necessary for γ-secretase activity. RNAi of PEN-2 abolished endoproteolytic cleavage of PS1, whereas overexpression of PEN-2 elevated the level of processed PS1 by-products, suggesting a primary role for PEN-2 in PS1 endoproteolysis. RNAi of APH-1 diminished the accumulation of PS1 resulting from PEN-2 RNAi and overexpression of APH-1 facilitated PEN-2-mediated PS1 proteolysis, indicating a more facilitative role for this cofactor [108].

A complementary approach to RNAi is to induce expression of specific genes in transgenic cell lines, thereby allowing one to assess the effects of increased expression and/or activity of individual genes. Application of overexpression and knock-down studies, when coupled to relevant *in vitro* assays, can be a powerful tool for functional validation. For example, several *in vitro* assays have been developed to study phenotypic markers of AD, such as hyperphosphorylation of tau protein and production of β-amyloid peptides [109]. Measurement of these fundamental molecular pathways provides insights into the formation of neurofibrillary tangles and amyloid plaques, two pathologies that are intimately involved in the development of AD. Importantly, with the availability of the entire human genome sequence and rapid proliferation of genomics technologies, high-throughput functional validation assays will be required to interpret the results of genomics experiments. The above *in vitro* methods are likely to become increasingly important in the study of neurological diseases since they can be applied to the study of any disease for which one has a relevant cell line and phenotypic assay.

While RNAi and gene overexpression studies are valuable tools for functional validation in cellular models (*in vitro*), animal models provide the best *in vivo* measure of functional validation. Although model systems are common in other diseases, when no behavioral phenotype manifests, such as in obesity [110,111], diabetes [112,113], autoimmune disease [114,115] and cancer [116–120], models for neurological disorders are much more difficult to come by.

Animal models have been invaluable for validating new biomarkers for a variety of neurological diseases. Several animal models of AD, mostly mouse, have been generated to test biomarker validation. Although transgenic mice harboring overexpressing mutations in either partial [121–125] or whole [126,127] human βAPP or PS1/PS2 [128–130] alone are not sufficient to develop the level of necessary AD-like phenotype, double-transgenic animals with two homozygous mutations (APP/PS) have robust Aβ/amyloid deposition [131,132].

Investigators using these double-transgenic AD mice have demonstrated that mitochondrial dysfunction (hypo- and/or hypermetabolism of cytochrome oxidase activity in different regions of the brain) could be a potential biomarker for AD [133]. Despite advancements in the development of APP/PS double-transgenic AD mice, the development of the tau pathology concurrent with the Aβ/amyloid deposition has been an elusive phenotype. A recent investigation by Oddo has developed the first triple-transgenic mouse model by directly introducing two additional transgenes into the germline of an already genetically modified mouse [134]. This is the first known mouse model to display both the plaque and tangle pathology. This model has enabled researchers to investigate the series of events leading to AD *in vivo*. These investigations suggest that synaptic dysfunction, including long-term potentiation deficits, could be a biomarker for AD and that this phenomenon likely precedes plaque and tangle pathology, offering new insight into the mechanism of degeneration in this debilitating disease.

Of course, these models will only be utilized to their full potential when synergistically combined with other types of biomarker identification. For example, knockout mice have been used to test the efficacy of novel PET radioligands that are being tested as potential pharmacotherapeutic targets. For instance, an antipsychotic, the putative dopamine D2 and 5-HT_{2A} receptor antagonist M100907, was tested for binding efficiency to its receptors and shown to have a novel mechanism that occurred in the absence of D2 receptor binding [135]. Another group used this technology to measure the neurotransmitter concentration in response to treatment with another pharmaceutical D2 receptor antagonist, ^{11}C raclopride, to study dopamine release in schizophrenia in response to amphetamines [136]. Eckelman suggests that this symbiotic relationship could facilitate the process of drug discovery by several methods, including authenticating the drug localization method, establishing the transport efficiency of drugs to their intended targets, determining the saturability of receptor sites and/or measuring pharmaceutical half-life [137].

In an alternative application, Dickey and coworkers used the APP/PS mice in combination with microarray analyses to identify genes that have altered expression in Aβ/amyloid deposition [138]. Using this approach they identified several genes associated with long-term potentiation and memory formation that showed decreased expression in the absence of altered expression of known presynaptic markers. These analyses would suggest that, in contrast to a previous study [134], synaptic dysfunction is not necessarily important for development of an AD-like pathology. However, this example highlights the factors that need to be addressed in future investigations, including standardization of protocols to ensure proper interpretation of the results. In the above example with apparently contradictory results, it is important to note that the mouse strains used are significantly different, one containing both amyloid and tau pathology [134] and the other containing only amyloid pathology [138]. This difference could suggest that tau pathology, present in the triple-transgenic, is responsible for the synaptic dysfunction not detected in the APP/PS mice.

Studies capitalizing on the use of animal models are currently underway in a variety of neurological diseases including depression [139], amyotrophic lateral sclerosis [140], PD [141] and MS [142]. However, not all animal models are genetically modified. Some, as in the case of amphetimine-induced mania in bipolar disorder [143], ketamine- [144] or ventral hippocampal lesion-induced schizophrenia [145,146], and MPTP/probenecid-induced PD, are chemically modified [147]. The continued use and improvement of these animal models are essential to the continued advancement of methodologies permitting the identification of biomarkers.

Additionally, validation of human biomarkers of disease is flawed when using an *in vitro* or rodent model, as exemplified by the apparent difficulty in mimicking the dramatic histopathology of humans. This demonstrates the need of ultimately performing retrospective and prospective diagnostic clinical studies to cement the accuracy, sensitivity and specificity of any biomarker for a clinical phenotype.

Advances in biomarker identification

In addition to the rapid expansion of neuroimaging in biomarker identification, new genetic and genomic tools have revolutionized the way in which neurologic diseases are investigated. Genotyping vast numbers of genetic polymorphisms in large populations is increasingly important for the identification of etiologically relevant mutations. Many of these etiologically relevant mutations have been discovered in response to monumental advances in high-throughput genome screening techniques that have been made in recent years. High-throughput screening started concurrent to the Human Genome Project. With this multi-institutional undertaking, the DNA sequences for humans and other common model organisms have and are being generated at an unprecedented rate. The release of the complete human genome sequence has precipitated vast numbers of investigations incorporating human genome sequence data into diverse applications including the previously mentioned models of structural and functional brain mapping via GENSAT, the Allen Brain Atlas and Neurome.

Another analysis that has revolutionized the identification of biomarkers is positional cloning. Positional cloning is a strategy for identifying genes that are etiological in nature, based upon their location within the genome. This is generally accomplished through genetic mapping but can also be achieved by cytogenetic visualization of chromosomal abnormalities, as was the case with Duchenne muscular dystrophy [148]. After physical mapping of the region, which necessitates the identification of gene content in that interval of the genome, functional candidate genes are sequenced to identify etiological sequence variants. Microarray technologies, including expression arrays, exon arrays, single nucleotide polymorphism (SNP) arrays and sequencing arrays, have recently come to play a role as adjuncts to physical mapping and identification of gene targets associated with disease [149].

Concurrent with the benefits of neuroimaging in diagnosis of neurologic disorders, there is the added benefit of being

able to look at early, predisease states of the brain and assess longitudinally the effects of the disease over time. It should be noted that although diagnostic utility of neuroimaging is increasingly important, the ultimate goal of neuroimaging in neurologic disorders is to identify physical changes in the brain that can be used as surrogate markers (e.g., quantification of blood glucose in diabetes) for the pathologic changes that bring about the disease (i.e., biomarkers).

Existing biomarkers in neurological disease

DeKosky and Marek have divided biomarkers into four categories: clinical, neuroimaging, genetic and biochemical [150]. In addition to established and evolving clinical diagnostic measures, such as psychometric and/or neuropsychological testing, the identification of specific genetic and protein biomarkers is rapidly accelerating. Although a small number of general imaging studies have demonstrated gross metabolic/structural changes, most of these have not been shown to be diagnostic or prognostic.

In contrast, genetic strategies have already yielded a number of AD-specific biomarkers. To date, most of the genetic research on AD has focused on four confirmed factors contributing to the inherited form of the disease, and all of these factors have been shown to increase the production and/or deposition of amyloid β-protein in the brain [151]. Mutations in the presenilin genes (PS1 and PS2) lead to the most aggressive form of familial, autosomal dominant AD, with at least 75 missense mutations in PS1 and three in PS2 shown to result in early-onset AD [152]. The presenilin mutations lead to altered cleavage of the β-amyloid precursor protein (βAPP) by γ-secretase, resulting in a twofold elevation of $A\beta_{42}$ in cultured skin fibroblasts [153] and a 1.5- to threefold increase in $A\beta_{42}$ in amyloid plaques from post-mortem brains of familial AD patients compared with sporadic AD patients [154,155]. Recent research suggests that presenilin itself may actually carry out the γ-secretase activity that cleaves βAPP [156].

The missense mutations are substantially more rare within the βAPP gene itself. These mutations, located at or near sites of secretase (α, β and/or γ) cleavage, lead to AD by altering proteolytic processing at these secretase sites that enhances cleavages that generate Aβ and promote amyloidogenesis. Nine βAPP missense mutations have been identified so far.

Last but not least, of the four confirmed AD-associated gene factors is the ε4 allele of the apolipoprotein E (ApoE4) gene. ApoE4 is, at present, the most significant predictor of outcome for sporadic AD. Inheritance of one or two copies of the ε4 allele increases the probability of developing AD and lessens mean age of onset in a dose-dependent manner compared with those carrying the ε2 or ε3 allele [157,158]. ApoE4 appears to augment the steady-state levels of Aβ (particularly $A\beta_{40}$) by diminishing its elimination from the brain [159]. It should be noted that the presence of an ε4 allele is a contributing, not causal, factor in the etiology of AD, as some humans homozygous for the ε4 allele show no AD symptoms and some with no ε4 alleles still develop the disease.

It is certain that other genetic factors mediating the etiology of AD exist and several new candidate genes have been identified. Alterations in or near the α_2-macroglobulin (α_2M) gene have been shown to segregate with the AD phenotype in some late-onset subjects [160]. In recent years, mutations in the hemochromatosis gene [161], ATP-binding cassette transporter A1 gene [162], Down's syndrome critical region 1 gene [163], the serotonin transporter gene [164] and several others have all been implicated in the etiology of AD. A comprehensive review of existing biomarkers for AD has been published by the Biological Markers Working Group and many more of these potential candidate genes are certain to emerge in the months and years to come [165].

Advances in genomics have made the investigation of genes associated with AD much more robust. Specifically, microarray technologies have allowed the assessment of thousands of genes at a time under different AD-related conditions. Recent research using microarrays of various types has identified hundreds of genes that are selectively up- or downregulated in patients displaying a range of AD symptomologies [166–168].

Although not as far advanced as AD, research into genetic markers for schizophrenia is also on the rise. Several biomarkers, many of them already independently reproduced, have been identified in just the last 5 years. SNPs in the neuregulin 1 [169,170], dysbindin [171,172], regulator of G-protein signaling-4 [173–175] and especially catechol-O-methyltransferase [176–179] genes have been replicated and are all good candidates for schizophrenia biomarkers. In addition, two of these potential loci already have relevant animal models that are currently being tested [169,176]. Though there are most certainly other genetic markers that have been associated with schizophrenia in the 100 years of investigation that this disease has undergone, few of the candidates have been successfully reproduced. It is hoped that these new potential markers will be further confirmed and lead to the types of therapeutic advancements that have been made in neurodegenerative disorders such as AD and PD.

Expert opinion

The identification of new biomarkers for neurological disease is essential for the continued advancement of clinical diagnostics and therapeutics. However, due to the inherent complexities involved in brain-related research, there are also intrinsic challenges. Four basic challenges in the identification of biomarkers in neurological disease were discussed in this review. These include:

- Availability of tissue at the site of pathology
- Poor clinical diagnostics and the extent of disease progression at time of diagnosis
- Complexity of the brain/tissue heterogeneity
- Lack of functional end points and models for validation studies

Several ways to overcome these basic challenges were also presented in the framework of one neurological disorder, AD. In short, advanced brain banking protocols will enable researchers to have access to high-quality tissues at the site(s)

of pathology. Highly developed imaging technologies can be used to improve diagnostics and therefore, permit clinicians to recognize and circumvent the rapid disease progression that is characteristic of these types of disorders. Sophisticated microscopy methodologies can be utilized to tease apart the innate heterogeneity that exists in an organ as complex as the brain by isolating individual neurons for examination. Lastly, several novel techniques for functional end-point identification and validation were presented.

Combining these new state-of-the-art strategies with the rapidly evolving strides in clinical diagnostic, genetic and therapeutic paradigms will enable a more rigorous diagnostic classification based on the identification of new and more efficacious biomarkers. These techniques will continue to reshape the research and development strategies for drugs, vaccines and novel gene therapies. The use of high-throughput screening technologies (such as cell- and tissue-based microarrays and mass spectrometry-based proteomic scanning) has rapidly accelerated the quantity and quality of molecular factors entering the drug development conduit. This has resulted in an increasing number of therapeutic targets, which necessitates new validation paradigms to ensure that these new therapeutics make it from the laboratory bench to the patient bedside.

In an effort to combine these various technologies to create a better, more sensitive assay, the Translational Genomics Research Institute (TGen) has undertaken a large, multi-institutional investigation into the etiology of AD. This National Institute on Aging-funded project will utilize the most up-to-date brain imaging techniques to identify regions of interest based on metabolic changes associated with the disease state. Once identified, individual neurons from these regions will be isolated from well-characterized patient samples by LCM and expression profiling of isolated RNA will be performed. Finally, RNAi will be utilized for functional validation of the resulting gene lists.

Another project being undertaken by TGen, which illustrates the scope of what the new high-throughput technologies can offer to biomarker identification in neurological disease, is a large whole-genome association study. This project is designed to look at almost every variable within the human genome and correlate it to a phenotype. It is to be performed in thousands of cases and compared with controls. This is a striking example of a revolutionary new approach to the identification of common pathogenic variants or combinations of variants that are associated with neurological disease.

Five-year view

In order for biomarkers to be utilized to their full potential, the key components of the different available technologies should be combined. Thus, standardization of diagnostic protocols to include the addition of neuroimaging and individual genotyping for the identification of genetic/genomic biomarkers to the customary clinical protocols of today is essential to achieving better precision in disease diagnostics. In addition, the constant advances in neuroimaging will eventually lead to their use as a more reliable diagnostic tool. This increased diagnostic efficacy should allow therapeutic intervention before more significant pathologies develop and, thus, result in more effectual treatments for these patients who have, as of yet, had little relief from these devastating diseases.

In addition to its role in clinical diagnostics, neuroimaging data will likely be used to identify specific biomarkers in the future. The development of specific ligands that target defined pathologies is of the utmost importance and will revolutionize biomarker identification. For instance, research is underway to develop specific ligands that target microglial activation, a pathological change believed to occur in the early stages of AD and PD [180]. In addition, a novel amyloid-imaging PET tracer, Pittsburgh compound B (PIB), has been shown to provide quantitative information on amyloid deposits in living subjects [181]. This technique appears to accurately and consistently detect amyloid deposition longitudinally in patients from the early stages of AD through conversion to full Alzheimer's dementia. Although the sample group was small, the results of this preliminary study suggest that this compound may constitute the first true biomarker derived solely from imaging data.

Additionally, 5 years will bring to the forefront mass spectrometry protocols, which are still in their infancy at present. Matrix-assisted laser desorption ionization with time of flight detectors (MALDI-TOF) and surface-enhanced laser desorption/ionization (SELDI-TOF) methods of mass spectrometry from serum and CSF are going to revolutionize the way biomarkers can be identified. These new technologies should generate rapid protein expression profiles of the more than half a million proteins or protein variants isolated from any of a variety of tissue samples. It can and will in the future be used for biomarker identification and diagnostics, as well as the study of protein–protein and protein–DNA interactions. In addition, its versatility will likely permit its use as a functional validation mechanism for potential biomarkers identified through other high-throughput techniques. For instance, the products of individual genes derived from gene lists generated via microarrays can be functionally validated on a MALDI- or SELDI-TOF protein chip to elucidate whether the altered messenger RNA expression pattern is translated into altered protein expression or is masked by some post-transcriptional, post-translational or RNA-stabilization mechanism.

Clinical diagnoses will be increasingly refined and subclassifications of common diseases will most certainly occur as we increase our ability to discover genetic and histologic variants. In addition, some diseases that are thought to be distinct may actually contain overlapping pathological features. Thus, it is likely that new classifications, such as synucleinopathies and/or tauopathies, will be used in the future to describe what could conceivably become a continuum of neurodegenerative disease, analogous to that being debated in neuropsychiatric disease in relation to bipolar disorder and schizophrenic psychoses [182–184].

The future of functional validation lies in the hands of novel high-throughput assays that can precisely and reproducibly analyze the huge amounts of data that are being generated by current and future experimental methodologies. Existing array-based chip technologies are advancing at an astounding rate. For example, the 10K SNP chip (Affymetrix) that can analyze more than 10,000 known human SNPs simultaneously was debuted at the European Society for Human Genetics Conference in Birmingham, UK in 2003. It is now possible to assay an estimated 1.5 million SNPs at a time using a new wafer-based technology from Perlegen, Inc. (Mountain View). New developments in SNP technology include a 250K SNP array anchored in the HapMap [204], an internationally developed haplotype map of the human genome that will describe the common patterns of human DNA sequence variation and will be available in the next few years. Another recent development in SNP typing is under development by Illumina. It attempts to combine the use of fiber optic bundles and specially coated beads that self assemble into an array. There can be up to 1500 different types in each individual bundle, with each bead containing oligonucleotides for a specific sequence. DNA from individual samples bind their complementary oligonucleotide on the coated bead permitting the tens of thousands of SNPs to be assayed at the same time [185]. These rapidly advancing technologies will continue to expand our knowledge of the human genome and help direct our search for efficacious biomarkers.

With these kinds of assays generating enormous amounts of data, functional validation becomes the bottleneck in the process of biomarker identification. Thus, it is likely that the future of functional validation rests in the capable hands of the siRNA technologies. With the completion of the Human Genome Project, the development of siRNAs that will silence every gene in the genome is currently underway. Once generated, the applications for this type of assay are boundless and will undoubtedly revolutionize the speed at which the vast amounts of data generated by the new, high-throughput chip technologies can be validated. *In vitro* analyses are currently fairly laborious and time consuming. Thus, the key will be to develop systems that will allow the *in vitro* siRNA assays to be employed in a high-throughput manner in order to keep up with the amount of data that will be generated by the data-generating infrastructure.

Lastly, there will be a great need for integrated databasing solutions to catalog and analyze the vast amounts of data that will be generated for these complex systems. The creation of a standardized protocol for brain banking guidelines used for collection, dissection and preservation of tissue samples is the key to achieving the level of tissue availability that is needed to fulfill the research demands of the future. In order to accommodate these research needs, the banking protocols and procedures must be designed for a broad set of experimental approaches. This may include significantly decreasing the PMI of many brain collections, implementing multiple methods of preservation (e.g., freezing and various fixation protocols) and thorough characterization and databasing of donors and specimens. Ideally, all data collected from primary research of banked materials should be routed back to the brain banking organization for assimilation into an ever-expanding database. Although data sharing can be a controversial topic with respect to publication, intellectual property rights and data access, one example of this, the National Alzheimer's Coordinating Center, is already utilizing this type of data-sharing strategy to facilitate collaborative research among its investigators. The creation of a centralized repository for archived tissues concurrent with a national database network of ante and post-mortem characterization of each donor and specimen that can be securely accessed by researchers via the internet would enable researchers across the globe to have access to the best possible samples for their respective experiments.

Although an extremely valuable resource, systems such as the National Center for Biotechnology Information/Genbank RNA, DNA and protein sequence database [205], will need to be associated with newer databasing paradigms such as the NINDS/National Institute of Mental Health array consortium [206], housed at the TGen. This network facilitates the acquisition and dissemination of high-quality expression profiling array data to many researchers, by establishing, cataloging and perhaps eventually standardizing the way in which this data is accessed. These types of databases will likely be established for the various types of data output (e.g., expression profiles, SNP genotypes and protein profiles) as well as for individual diseases (e.g., AD and schizophrenia). In addition, these databases, new and existing, will have to be expanded to include other types of data that will be garnered from the new methodologies that will be employed in next 5 years.

Thus, a functional paradigm that might reflect the general course of neurological disease biomarker identification in the next 5 years can be summarized as follows:

- Analysis and dissection of highly complex, heterogenous tissue samples using high-performance imaging systems (e.g., LCM)
- Examination of cellular macromolecules (e.g., RNA, DNA and protein) via some high-throughput chip-based mechanism (SNP typing, microarray, MALDI- and/or SELDI-TOF mass spectrometry)
- Automated data generation and archiving
- Data extraction
- Integration of all data into internet-based information management systems

High-throughput strategies such as this will successfully ensure that the identification of biomarkers in neurological diseases can continue to advance. It will also facilitate further development of novel diagnostics and therapeutics to aid the patients and families that are suffering from these tragic neurological diseases.

Key issues

- Identification of biomarkers is particularly challenging in neurologic diseases due to a variety of factors, including availability of tissue at the site of pathology, poor clinical diagnostics (clinical heterogeneity), complexity of the brain (tissue heterogeneity) and lack of functional end points and models for validation.
- Advances in research technology, including brain banking, brain imaging, laser capture microdissection and online databases (e.g., GENSAT and Allen Brain Atlas), high-throughput genomics and new functional validation techniques such as RNA interference can help to overcome these challenges.
- New databasing protocols are necessary to accommodate the vast amounts of data generated by the new high-throughput genomics and brain-imaging technologies.
- Access to generated data is critical to continued progress in biomarker identification so that this information can be used to devise new diagnostics and therapeutics that will help people suffering from these insidious diseases.

References

Papers of special note have been highlighted as:
- • of interest
- •• of considerable interest

1 Atkinson AEA. Biomarkers and surrogate end points: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* 69(3), 89–95 (2001).

2 Gershon ES, Goldin LR. Clinical methods in psychiatric genetics. I. Robustness of genetic marker investigative strategies. *Acta Psychiatr. Scand*. 74(2), 113–118 (1986).

3 Leboyer M, Bellivier F, Nosten-Bertrand M *et al.* Psychiatric genetics: search for phenotypes. *Trends Neurosci*. 21(3), 102–105 (1998).

4 Hulette CM, Earl NL, Crain BJ. Evaluation of cerebral biopsies for the diagnosis of dementia. *Arch. Neurol*. 49(1), 28–31 (1992).

5 Perry EK, Perry RH, Tomlinson BE. The influence of agonal status on some neurochemical activities of postmortem human brain tissue. *Neurosci. Lett*. 29(3), 303–307 (1982).

6 Ferrer I, Blanco R. N-myc and c-myc expression in Alzheimer's disease, Huntington's disease and Parkinson's disease. *Brain Res. Mol. Brain Res.* 77(2), 270–276 (2000).

7 McKee AC. Brain banking: basic science methods. *Alzheimer Dis. Assoc. Disord*. 13(Suppl. 1), S39–S44 (1999).

8 Sarris M, Garrick TM, Sheedy D, Harper CG. Banking for the future: an Australian experience in brain banking. *Pathology* 34(3), 225–229 (2002).

9 Perry EK, Perry RH. Human brain neurochemistry – some postmortem problems. *Life Sci*. 33(18), 1733–1743 (1983).

10 Hock C, Heese K, Hulette C, Rosenberg C, Otten U. Region-specific neurotrophin imbalances in Alzheimer's disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch. Neurol*. 57(6), 846–851 (2000).

11 Albers DS, Augood SJ, Martin DM *et al.* Evidence for oxidative stress in the subthalamic nucleus in progressive supranuclear palsy. *J. Neurochem*. 73(2), 881–884 (1999).

12 Hulette CM, Welsh-Bohmer KA, Crain B *et al.* Rapid brain autopsy. The Joseph and Kathleen Bryan Alzheimer's Disease Research Center experience. *Arch. Pathol. Lab. Med*. 121(6), 615–618 (1997).

13 Haroutunian V, Davis KL. Issues and perspectives on brain tissue banking. *Curr. Psychiatry Rep*. 4(4), 233–234 (2002).

14 Riaz S, Nowack WJ. Diagnostic problems in multiple sclerosis: overreliance on neuroimaging. *South Med. J*. 91(3), 270–272 (1998).

15 Levin N, Mor M, Ben-Hur T. Patterns of misdiagnosis of multiple sclerosis. *Isr. Med. Assoc. J*. 5(7), 489–490 (2003).

16 Poewe W, Wenning G. The differential diagnosis of Parkinson's disease. *Eur. J. Neurol*. 9(Suppl. 3), 23–30 (2002).

17 Mangino M, Middlemiss C. Alzheimer's disease: preventing and recognizing a misdiagnosis. *Nurse Pract*. 22(10), 58–59, 63–58, 70, (1997).

18 McKhann G, Drachman D, Folstein M *et al.* Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34(7), 939–944 (1984).

19 American Psychiatric Association. Task Force on DSM-IV. *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV.* American Psychiatric Association, DC, USA (1994).

20 Chui H, Young Lee A. *Clinical Criteria for Various Dementia Subtypes: Alzheimer's disease (AD), Vascular Dementia (VaD), Dementia with Lewy Bodies (DLB) and Frontotemporal Dementia (FTD).* Qizilbash N, Schneider L, Chui H *et al.* (Eds), Blackwell Science, Oxford UK (2001).

21 Petrovitch H, White LR, Ross GW *et al.* Accuracy of clinical criteria for AD in the Honolulu-Asia Aging Study, a population-based study. *Neurology* 57(2), 226–234 (2001).

22 Varma AR, Snowden JS, Lloyd JJ *et al.* Evaluation of the NINCDS-ADRDA criteria in the differentiation of Alzheimer's disease and frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* 66(2), 184–188 (1999).

23 Gearing M, Mirra SS, Hedreen JC *et al.* The Consortium to establish a registry for Alzheimer's disease (CERAD). Part X. Neuropathology confirmation of the clinical diagnosis of Alzheimer's disease. *Neurology* 45(3 Pt 1), 461–466 (1995).

24 Suslow T, Roestel C, Ohrmann P, Arolt V. The experience of basic emotions in schizophrenia with and without affective negative symptoms. *Compr. Psychiatry* 44(4), 303–310 (2003).

25 Young DA, Campbell Z, Zakzanis KK, Weinstein E. A comparison between an interview and a self-report method of insight assessment in chronic schizophrenia. *Schizophr. Res*. 63(1–2), 103–109 (2003).

26 Garvey MJ, Tuason VB. Mania misdiagnosed as schizophrenia. *J. Clin. Psychiatry* 41(3), 75–78 (1980).

27 Hurley AD. The misdiagnosis of hallucinations and delusions in persons with mental retardation: a neurodevelopmental perspective. *Semin. Clin. Neuropsychiatry* 1(2), 122–133 (1996).

28 Lisanby SH, Kohler C, Swanson CL, Gur RE. Psychosis secondary to brain tumor. *Semin. Clin. Neuropsychiatry* 3(1), 12–22 (1998).

29 Bottlender R, Sato T, Groll C *et al.* Negative symptoms in depressed and schizophrenic patients: how do they differ? *J. Clin. Psychiatry* 64(8), 954–958 (2003).

30 Ghaemi N, Sachs GS, Goodwin FK. What is to be done? Controversies in the diagnosis and treatment of manic-depressive illness. *World J. Biol. Psychiatry* 1(2), 65–74 (2000).

31 Farina E, Pomati S, Mariani C. Observations on dementias with possibly reversible symptoms. *Aging*, 11(5), 323–328 (1999).

32 Knopman DS, DeKosky ST, Cummings JL *et al.* Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 56(9), 1143–1153 (2001).

33 Gado M, Hughes CP, Danziger W *et al.* Volumetric measurements of the cerebrospinal fluid spaces in demented subjects and controls. *Radiology* 144(3), 535–538 (1982).

34 Kelly CA, Harvey RJ, Cayton H. Drug treatments for Alzheimer's disease. *Br. Med. J.* 314(7082), 693–694 (1997).

35 Larson EB. New drug treatments for Alzheimer's disease. *Arch. Intern. Med.* 158(9), 941–942 (1998).

36 Scahill RI, Schott JM, Stevens JM, Rossor MN, Fox NC. Mapping the evolution of regional atrophy in Alzheimer's disease: unbiased analysis of fluid-registered serial MRI. *Proc. Natl Acad. Sci. USA* 99(7), 4703–4707 (2002).

37 Jack CR Jr, Dickson DW, Parisi JE *et al.* Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. *Neurology* 58(5), 750–757 (2002).

38 de Leon M, Bobinski M, Convit A, Wolf O, Insausti R. Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. *Neurology* 56(6), 820–821 (2001).

39 Fox NC, Freeborough PA, Rossor MN. Visualisation and quantification of rates of atrophy in Alzheimer's disease. *Lancet* 348(9020), 94–97 (1996).

40 Fox NC, Crum WR, Scahill RI *et al.* Imaging of onset and progression of Alzheimer's disease with voxel-compression mapping of serial magnetic resonance images. *Lancet* 358(9277), 201–205 (2001).

41 Chen K, Reiman E, Alexander G, Bandy D, Renaut R. An automated algorithm for the computation of brain volume change from sequential MRIs using an iterative principal component analysis and its evaluation for the assessment of whole brain atrophy rates in patients with probable Alzheimer's disease. *Neuroimage* (2004) (In Press).

42 Christensen GE, Rabbitt RD, Miller MI. Deformable templates using large deformation kinematics. *IEEE Trans. Image Process*. 5(10), 1435–1144 (1996).

43 Chetelat G, Desgranges B, De La Sayette V *et al.* Mapping gray matter loss with voxel-based morphometry in mild cognitive impairment. *Neuroreport* 13(15), 1939–1943 (2002).

44 Rusch N, van Elst LT, Ludaescher P *et al.* A voxel-based morphometric MRI study in female patients with borderline personality disorder. *Neuroimage* 20(1), 385–392 (2003).

45 Scheltens P, Fox N, Barkhof F, De Carli C. Structural magnetic resonance imaging in the practical assessment of dementia: beyond exclusion. *Lancet Neurol*. 1(1), 13–21 (2002).

46 Chan D, Fox NC, Scahill RI *et al.* Patterns of temporal lobe atrophy in semantic dementia and Alzheimer's disease. *Ann. Neurol*. 49(4), 433–442 (2001).

47 Galton CJ, Gomez-Anson B, Antoun N *et al.* Temporal lobe rating scale: application to Alzheimer's disease and frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* 70(2), 165–173 (2001).

48 Roman GC, Tatemichi TK, Erkinjuntti T *et al.* Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 43(2), 250–260 (1993).

49 Silverman DH, Small GW, Chang CY *et al.* Positron emission tomography in evaluation of dementia: regional brain metabolism and long-term outcome. *JAMA* 286(17), 2120–2127 (2001).

50 Drzezga A, Lautenschlager N, Siebner H *et al.* Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: a PET follow-up study. *Eur. J. Nucl. Med.* 30(8), 1104–1113 (2003).

51 Jagust WJ, Budinger TF, Reed BR. The diagnosis of dementia with single photon emission computed tomography. *Arch. Neurol*. 44(3), 258–262 (1987).

52 Holman BL, Tumeh SS. Single-photon emission computed tomography (SPECT). Applications and potential. *JAMA* 263(4), 561–564 (1990).

53 Ettinger U, Chitnis XA, Kumari V *et al.* Magnetic resonance imaging of the thalamus in first-episode psychosis. *Am. J. Psychiatry* 158(1), 116–118 (2001).

54 Kuperberg GR, Broome MR, McGuire PK *et al.* Regionally localized hinning of the cerebral cortex in schizophrenia. *Arch. Gen. Psychiatry* 60(9), 878–888 (2003).

55 Milev P, Ho BC, Arndt S, Nopoulos P, Andreasen NC. Initial magnetic resonance imaging volumetric brain measurements and outcome in schizophrenia: a prospective longitudinal study with 5-year follow-up. *Biol. Psychiatry* 54(6), 608–615 (2003).

56 Muller JL, Klein HE. Neuroleptic therapy influences basal ganglia activation: a functional magnetic resonance imaging study comparing controls to haloperidol- and olanzapine-treated in-patients. *Psychiatry Clin. Neurosci*. 54(6), 653–658 (2000).

57 Scheepers FE, de Wied CC, Hulshoff Pol HE *et al.* The effect of clozapine on caudate nucleus volume in schizophrenic patients previously treated with typical antipsychotics. *Neuropsychopharmacology* 24(1), 47–54 (2001).

58 Johnstone EC, Crow TJ, Frith CD, Husband J, Kreel L. Cerebral ventricular size and cognitive impairment in chronic schizophrenia. *Lancet* 2(7992), 924–926 (1976).

59 Weinberger DR, DeLisi LE, Neophytides AN, Wyatt RJ. Familial aspects of CT scan abnormalities in chronic schizophrenic patients. *Psychiatry Res*. 4(1), 65–71 (1981).

60 Reveley AM, Reveley MA, Clifford CA, Murray RM. Cerebral ventricular size in twins discordant for schizophrenia. *Lancet* 1(8271), 540–541 (1982).

61 DeLisi LE, Goldin LR, Hamovit JR *et al.* A family study of the association of increased ventricular size with schizophrenia. *Arch. Gen. Psychiatry* 43(2), 148–153 (1986).

62 Ketter TA, Kimbrell TA, George MS *et al.* Effects of mood and subtype on cerebral glucose metabolism in treatment-resistant bipolar disorder. *Biol. Psychiatry* 49(2), 97–109 (2001).

63 Strakowski SM, DelBello MP, Zimmerman ME *et al.* Ventricular and periventricular structural volumes in first- vs. multiple-episode bipolar disorder. *Am. J. Psychiatry* 159(11), 1841–1847 (2002).

64 Thompson PM, Vidal C, Giedd JN *et al.*. Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. *Proc. Natl Acad. Sci. USA* 98(20), 11650–11655 (2001).

65 Kubicki M, Shenton ME, Salisbury DF *et al.* Voxel-based morphometric analysis of gray matter in first episode schizophrenia. *Neuroimage* 17(4), 1711–1719 (2002).

66 Schlosser R, Gesierich T, Kaufmann B *et al.* Altered effective connectivity during working memory performance in schizophrenia: a study with fMRI and structural equation modeling. *Neuroimage* 19(3), 751–763 (2003).

67 Benson BE, Willis MW, Ketter TA *et al.* Altered relationships in RCMRGLU associativity in bipolar and unipolar illness. *Biological Psychiatry* 47(1), S108 (2000).

68 Heinz A, Jones DW, Raedler T *et al.* Neuropharmacological studies with SPECT in neuropsychiatric disorders. *Nucl. Med. Biol.* 27(7), 677–682 (2000).

69 Xiberas X, Martinot JL, Mallet L *et al.* Extrastriatal and striatal D(2) dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. *Br. J. Psychiatry* 179, 503–508 (2001).

70 Tauscher-Wisniewski S, Kapur S, Tauscher J *et al.* Quetiapine: an effective antipsychotic in first-episode schizophrenia despite only transiently high dopamine-2 receptor blockade. *J. Clin. Psychiatry* 63(11), 992–997 (2002).

71 Gefvert O, Lindstrom LH, Waters N *et al.* Different corticostriatal patterns of L-DOPA utilization in patients with untreated schizophrenia and patients treated with classical antipsychotics or clozapine. *Scand. J. Psychol.* 44(3), 289–292 (2003).

72 Goodwin GM, Cavanagh JT, Glabus MF *et al.* Uptake of ^{99mT}c-exametazime shown by single photon emission computed tomography before and after lithium withdrawal in bipolar patients: associations with mania. *Br. J. Psychiatry* 170, 426–430 (1997).

73 Schlosser R, Schlegel S, Hiemke C *et al.* [123I]IBZM SPECT in patients treated with typical and atypical neuroleptics: relationship to drug plasma levels and extrapyramidal side effects. *Psychiatry Res.* 75(2), 103–114 (1997).

74 Pazzaglia PJ, Post RM, Ketter TA *et al.* Nimodipine monotherapy and carbamazepine augmentation in patients with refractory recurrent affective illness. *J. Clin. Psychopharmacol.* 18(5), 404–413 (1998).

75 Yatham LN, Liddle PF, Lam RW *et al.* PET study of the effects of valproate on dopamine D(2) receptors in neuroleptic- and mood-stabilizer-naive patients with nonpsychotic mania. *Am. J. Psychiatry* 159(10), 1718–1723 (2002).

76 Manji HK, Moore GJ, Chen G. Lithium upregulates the cytoprotective protein Bcl-2 in the CNS *in vivo*: a role for neurotrophic and neuroprotective effects in manic depressive illness. *J. Clin. Psychiatry* 61(Suppl. 9), 82–96 (2000).

77 Moore GJ, Bebchuk JM, Wilds IB *et al.* Lithium-induced increase in human brain grey matter. *Lancet* 356(9237), 1241–1242 (2000).

78 Riehemann S, Volz HP, Stutzer P *et al.* Hypofrontality in neuroleptic-naive schizophrenic patients during the Wisconsin Card Sorting Test–a fMRI study. *Eur. Arch. Psychiatry Clin. Neurosci.* 251(2), 66–71 (2001).

79 Barch DM, Csernansky JG, Conturo T, Snyder AZ. Working and long-term memory deficits in schizophrenia: is there a common prefrontal mechanism? *J. Abnorm. Psychol.* 111(3), 478–494 (2002).

80 Perlstein WM, Dixit NK, Carter CS, Noll DC, Cohen JD. Prefrontal cortex dysfunction mediates deficits in working memory and prepotent responding in schizophrenia. *Biol. Psychiatry* 53(1), 25–38 (2003).

81 Callicott JH, Bertolino A, Mattay VS *et al.* Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. *Cereb. Cortex* 10(11), 1078–1092 (2000).

82 Ramsey NF, Koning HA, Welles P *et al.* Excessive recruitment of neural systems subserving logical reasoning in schizophrenia. *Brain* 125(8), 1793–1807 (2002).

83 Quintana J, Wong T, Ortiz-Portillo E *et al.* Prefrontal-posterior parietal networks in schizophrenia: primary dysfunctions and secondary compensations. *Biol. Psychiatry* 53(1), 12–24 (2003).

84 Selemon LD, Rajkowska G. Cellular pathology in the dorsolateral prefrontal cortex distinguishes schizophrenia from bipolar disorder. *Curr. Mol. Med.* 3(5), 427–436 (2003).

85 Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol. Psychiatry* 7(4), 405–411 (2002).

86 Levinson DF, Levinson MD, Segurado R, Lewis CM. Genome scan meta-analysis of schizophrenia and bipolar disorder, part I: methods and power analysis. *Am. J. Hum. Genet.* 73(1), 17–33 (2003).

87 Lewis CM, Levinson DF, Wise LH *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am. J. Hum. Genet.* 73(1), 34–48 (2003).

88 Segurado R, Detera-Wadleigh SD, Levinson DF *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: bipolar disorder. *Am. J. Hum. Genet.* 73(1), 49–62 (2003).

89 Duda JE, Giasson BI, Mabon ME, Lee VM, Trojanowski JQ. Novel antibodies to synuclein show abundant striatal pathology in Lewy body diseases. *Ann. Neurol.* 52(2), 205–210 (2002).

90 Mori H, Oda M, Komori T *et al.* Lewy bodies in progressive supranuclear palsy. *Acta. Neuropathol.* 104(3), 273–278 (2002).

91 SantaCruz KS, Tasaki CS, Kim RC, Cotman CW. Brainstem and cortical Lewy bodies in patients presenting clinically with Alzheimer's disease. *J. Alzheimer's Dis.* 4(1), 11–17 (2002).

92 Braak H, Del Tredici K, Rub U *et al.* Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24(2), 197–211 (2003).

93 Marui W, Iseki E, Nakai T *et al.* Progression and staging of Lewy pathology in brains from patients with dementia with Lewy bodies. *J. Neurol. Sci.* 195(2), 153–159 (2002).

94 Hutton M, Lendon CL, Rizzu P *et al.* Association of missense and 5´-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393(6686), 702–705 (1998).

95 van de Warrenburg BP, Lammens M, Lucking CB *et al.* Clinical and pathologic abnormalities in a family with parkinsonism and parkin gene mutations. *Neurology* 56(4), 555–557 (2001).

96 Duda JE, Giasson BI, Mabon ME *et al.* Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. *Acta Neuropathol (Berl)* 104(1), 7–11 (2002).

97 Iseki E, Togo T, Suzuki K *et al.* Dementia with Lewy bodies from the perspective of tauopathy. *Acta. Neuropathol. (Berl)*, 105(3), 265–270 (2003).

98 Sanchez MP, Gonzalo I, Avila J, De Yebenes JG. Progressive supranuclear palsy and tau hyperphosphorylation in a patient with a C212Y parkin mutation. *J. Alzheimers Dis.* 4(5), 399–404 (2002).

99 Barker WW, Luis CA, Kashuba A *et al.* Relative frequencies of Alzheimer's disease, Lewy body, vascular and frontotemporal dementia and hippocampal sclerosis in the State of Florida Brain Bank. *Alzheimer Dis. Assoc. Disord*. 16(4), 203–212 (2002).

100 Mastaglia FL, Johnsen RD, Byrnes ML, Kakulas BA. Prevalence of amyloid-β deposition in the cerebral cortex in Parkinson's disease. *Mov. Disord*. 18(1), 81–86 (2003).

101 Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta. Neuropathol.* (*Berl*) 82(4), 239–259 (1991).

102 Luzzi V, Mahadevappa M, Raja R, Warrington JA, Watson MA. Accurate and reproducible gene expression profiles from laser capture microdissection, transcript amplification and high density oligonucleotide microarray analysis. *J. Mol. Diagn*. 5(1), 9–14 (2003).

103 Emmert-Buck MR, Bonner RF, Smith PD *et al.* Laser capture microdissection. *Science* 274(5289), 998–1001 (1996).

104 Gong S, Zheng C, Doughty ML *et al.* A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425(6961), 917–925 (2003).

105 Dykxhoorn DM, Novina CD, Sharp PA. Killing the messenger: short RNAs that silence gene expression. *Nature Rev. Mol. Cell Biol*. 4(6), 457–467 (2003).

106 Gitlin L, Karelsky S, Andino R. Short interfering RNA confers intracellular antiviral immunity in human cells. *Nature* 418(6896), 430–434 (2002).

107 Weiner A. Soaking up RNAi. *Mol. Cell* 12(3), 535–536 (2003).

108 Luo WJ, Wang H, Li H *et al.* PEN-2 and APH-1 co-ordinately regulate proteolytic processing of presenilin 1. *J. Biol. Chem*. 278(10), 7850–7854 (2003).

109 Lue LF, Walker DG. Modeling Alzheimer's disease immune therapy mechanisms: interactions of human postmortem microglia with antibody-opsonized amyloid β peptide. *J. Neurosci. Res.* 70(4), 599–610 (2002).

110 Takahashi K, Suwa H, Ishikawa T, Kotani H. Targeted disruption of H3 receptors results in changes in brain histamine tone leading to an obese phenotype. *J. Clin. Invest*. 110(12), 1791–1799 (2002).

111 Williams T, Chambers J, Roberts L, Henderson R, Overton J. Diet-induced obesity and cardiovascular regulation in C57BL/6J mice. *Clin. Exp. Pharmacol. Physiol*. 30(10), 769–778 (2003).

112 Davies EL, Abdel-Wahab YH, Flatt PR, Bailey CJ. Functional enhancement of electrofusion-derived BRIN-BD11 insulin-secreting cells after implantation into diabetic mice. *Int. J. Exp. Diabetes Res.* 2(1), 29–36 (2001).

113 Lesniewski LA, Miller TA, Armstrong RB. Mechanisms of force loss in diabetic mouse skeletal muscle. *Muscle Nerve* 28(4), 493–500 (2003).

114 Taki S. Type I interferons and autoimmunity: lessons from the clinic and from IRF-2-deficient mice. *Cytokine Growth Factor Rev.* 13(4–5), 379–391 (2002).

115 Ishimaru N, Arakaki R, Watanabe M *et al.* Development of autoimmune exocrinopathy resembling Sjogren's syndrome in estrogen-deficient mice of healthy background. *Am. J. Pathol*. 163(4), 1481–1490 (2003).

116 Jackson EL, Willis N, Mercer K *et al.* Analysis of lung tumor initiation and progression using conditional expression of oncogenic *K-ras*. *Genes Dev.* 15(24), 3243–3248 (2001).

117 Li X, Wang J, An Z *et al.* Optically imageable metastatic model of human breast cancer. *Clin. Exp. Metastasis* 19(4), 347–350 (2002).

118 Cox C, Merajver SD, Yoo S *et al.* Inhibition of the growth of squamous cell carcinoma by tetrathiomolybdate-induced copper suppression in a murine model. *Arch. Otolaryngol. Head Neck Surg*. 129(7), 781–785 (2003).

119 Garcia-Castro J, Rio P, Lillo R *et al.* Purging of leukemia-contaminated bone marrow grafts using suicide adenoviral vectors: an *in vivo* murine experimental model. *Gene Ther.* 10(16), 1328–1335 (2003).

120 Sfacteria A, Bertani C, Costantino G *et al.* Cyclin D1 expression in pre-cancerous and cancerous lesions of the canine mammary gland. *J. Comp. Pathol*. 128(4), 245–251 (2003).

121 Yamaguchi F, Richards SJ, Beyreuther K *et al.* Transgenic mice for the amyloid precursor protein 695 isoform have impaired spatial memory. *Neuroreport* 2(12), 781–784 (1991).

122 Wirak DO, Bayney R, Ramabhadran TV *et al.* Deposits of amyloid β protein in the central nervous system of transgenic mice. *Science* 253(5017), 323–325 (1991).

123 Kammesheidt A, Boyce FM, Spanoyannis AF *et al.* Deposition of β/A4 immunoreactivity and neuronal pathology in transgenic mice expressing the carboxyl-terminal fragment of the Alzheimer amyloid precursor in the brain. *Proc. Natl Acad. Sci. USA* 89(22), 10857–10861 (1992).

124 Higgins LS, Holtzman DM, Rabin J, Mobley WC, Cordell B. Transgenic mouse brain histopathology resembles early Alzheimer's disease. *Ann. Neurol*. 35(5), 598–607 (1994).

125 Moran PM, Higgins LS, Cordell B, Moser PC. Age-related learning deficits in transgenic mice expressing the 751-amino acid isoform of human β-amyloid precursor protein. *Proc. Natl Acad. Sci. USA* 92(12), 5341–5345 (1995).

126 Lamb BT, Sisodia SS, Lawler AM *et al.* Introduction and expression of the 400 kilobase amyloid precursor protein gene in transgenic mice [corrected]. *Nature Genet*. 5(1), 22–30 (1993).

127 Pearson BE, Choi TK. Expression of the human β-amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice. *Proc. Natl Acad. Sci. USA* 90(22), 10578–10582 (1993).

128 Borchelt DR, Thinakaran G, Eckman CB *et al.* Familial Alzheimer's disease-linked presenilin 1 variants elevate A β1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron* 17(5), 1005–1013 (1996).

129 Citron M, Westaway D, Xia W *et al.* Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. *Nature Med*. 3(1), 67–72 (1997).

130 Chui DH, Tanahashi H, Ozawa K *et al.* Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nature Med*. 5(5), 560–564 (1999).

131 Borchelt DR, Ratovitski T, van Lare J *et al.* Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* 19(4), 939–945 (1997).

132 Holcomb L, Gordon MN, McGowan E *et al.* Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Med*. 4(1), 97–100 (1998).

133 Strazielle C, Sturchler-Pierrat C, Staufenbiel M, Lalonde R. Regional brain cytochrome oxidase activity in β-amyloid precursor protein transgenic mice with the Swedish mutation. *Neuroscience* 118(4), 1151–1163 (2003).

134 Oddo S, Caccamo A, Shepherd JD *et al.* Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* 39(3), 409–421 (2003).

135 Offord SJ, Wong DF, Nyberg S. The role of positron emission tomography in the drug development of M100907, a putative antipsychotic with a novel mechanism of action. *J. Clin. Pharmacol.* Suppl, 17S–24S (1999).

136 Breier A, Su TP, Saunders R *et al.* Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc. Natl Acad. Sci. USA* 94(6), 2569–2574 (1997).

137 Eckelman WC. The use of PET and knockout mice in the drug discovery process. *Drug Discov. Today* 8(9), 404–410 (2003).

138 Dickey CA, Loring JF, Montgomery J *et al.* Selectively reduced expression of synaptic plasticity-related genes in amyloid precursor protein + presenilin-1 transgenic mice. *J. Neurosci*. 23(12), 5219–5226 (2003).

139 Bale TL, Vale WW. Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: sexually dichotomous responses. *J. Neurosci*. 23(12), 5295–5301 (2003).

140 Hamson DK, Hu JH, Krieger C, Watson NV. Lumbar motoneurone neurone neurone fate in a mouse model of amyotrophic lateral sclerosis. *Neuroreport* 13(17), 2291–2294 (2002).

141 Masliah E, Rockenstein E, Veinbergs I *et al.* Dopaminergic loss and inclusion body formation in α-synuclein mice: implications for neurodegenerative disorders. *Science* 287(5456), 1265–1269 (2000).

142 Werner P, Pitt D, Raine CS. Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. *Ann. Neurol*. 50(2), 169–180 (2001).

143 Lamberty Y, Margineanu DG, Klitgaard H. Effect of the new anti-epileptic drug levetiracetam in an animal model of mania. *Epilepsy Behav.* 2(5), 454–459 (2001).

144 Becker A, Peters B, Schroeder H *et al.* Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 27(4), 687–700 (2003).

145 Lipska BK, Lerman DN, Khaing ZZ, Weickert CS, Weinberger DR. Gene expression in dopamine and GABA systems in an animal model of schizophrenia: effects of antipsychotic drugs. *Eur. J. Neurosci.* 18(2), 391–402 (2003).

146 Wood GK, Quirion R, Srivastava LK. Early environment contributes to developmental disruption of MPFC after neonatal ventral hippocampal lesions in rats. *Synapse* 50(3), 223–232 (2003).

147 Meredith GE, Totterdell S, Petroske E *et al.* Lysosomal malfunction accompanies α-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Res.* 956(1), 156–165 (2002).

148 Bertelson CJ, Bartley JA, Monaco AP *et al.* Localisation of Xp21 meiotic exchange points in Duchenne muscular dystrophy families. *J. Med. Genet.* 23(6), 531–537 (1986).

149 Dobrin SE, Stephan DA. Integrating microarrays into disease-gene identification strategies. *Expert Rev. Mol. Diagn.* 3(3), 375–385 (2003).

150 DeKosky ST, Marek K. Looking backward to move forward: early detection of neurodegenerative disorders. *Science* 302(5646), 830–834 (2003).

• Review describing current neuroimaging strategies to improve diagnostics of neurodegenerative disorders.

151 Selkoe DJ. Alzheimer's disease: genotypes, phenotypes and treatments. *Science* 275(5300), 630–631 (1997).

152 Hardy J. The Alzheimer family of diseases: many etiologies, one pathogenesis? *Proc. Natl Acad. Sci. USA* 94(6), 2095–2097 (1997).

153 Scheuner D, Eckman C, Jensen M *et al.* Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Med.* 2(8), 864–870 (1996).

154 Lemere CA, Lopera F, Kosik KS *et al.* The E280A presenilin 1 Alzheimer mutation produces increased A β 42 deposition and severe cerebellar pathology. *Nature Med.* 2(10), 1146–1150 (1996).

155 Mann DM, Iwatsubo T, Cairns NJ *et al.* Amyloid β protein (Aβ) deposition in chromosome 14-linked Alzheimer's disease: predominance of Aβ42(43). *Ann. Neurol.* 40(2), 149–156 (1996).

156 Wolfe MS, Xia W, Ostaszewski BL *et al.* Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ- secretase activity. *Nature* 398(6727), 513–517 (1999).

157 Corder EH, Saunders AM, Strittmatter WJ *et al.* Gene dose of apolipoprotein E Type ε4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261(5123), 921–923 (1993).

158 Saunders AM, Strittmatter WJ, Schmechel D *et al.* Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 43(8), 1467–1472 (1993).

159 Gearing M, Mori H, Mirra SS. Aβ-peptide length and apolipoprotein E genotype in Alzheimer's disease. *Ann. Neurol.* 39(3), 395–399 (1996).

160 Blacker D, Wilcox MA, Laird NM *et al.* α-2 macroglobulin is genetically associated with Alzheimer's disease. *Nature Genet.* 19(4), 357–360 (1998).

161 Combarros O, Garcia-Roman M, Fontalba A *et al.* Interaction of the H63D mutation in the hemochromatosis gene with the apolipoprotein E epsilon 4 allele modulates age at onset of Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 15(3), 151–154 (2003).

162 Wollmer MA, Streffer JR, Lutjohann D *et al.* ABCA1 modulates CSF cholesterol levels and influences the age at onset of Alzheimer's disease. *Neurobiol. Aging* 24(3), 421–426 (2003).

163 Ermak G, Davies KJ. Gene expression in Alzheimer's disease. *Drugs Today (Barc)* 38(7), 509–516 (2002).

164 Hu M, Retz W, Baader M *et al.* Promoter polymorphism of the 5-HT transporter and Alzheimer's disease. *Neurosci. Lett.* 294(1), 63–65 (2000).

165 Frank RA, Galasko D, Hampel H *et al.* Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the Biological Markers Working Group: NIA initiative on neuroimaging in Alzheimer's disease. *Neurobiol. Aging* 24(4), 521–536 (2003).

166 Ho L, Guo Y, Spielman L *et al.* Altered expression of a-type but not b-type synapsin isoform in the brain of patients at high risk for Alzheimer's disease assessed by DNA microarray technique. *Neurosci. Lett.* 298(3), 191–194 (2001).

167 Hata R, Masumura M, Akatsu H *et al.* Upregulation of calcineurin A β mRNA in the Alzheimer's disease brain: assessment by cDNA microarray. *Biochem. Biophys. Res. Commun.* 284(2), 310–316 (2001).

168 Colangelo V, Schurr J, Ball MJ *et al.* Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor

downregulation and upregulation of apoptotic and pro-inflammatory signaling. *J. Neurosci. Res.* 70(3), 462–473 (2002).

169 Stefansson H, Sigurdsson E, Steinthorsdottir V *et al.* Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet*. 71(4), 877–892 (2002).

170 Stefansson H, Sarginson J, Kong A *et al.* Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am. J. Hum. Genet*. 72(1), 83–87 (2003).

171 Straub RE, Jiang Y, MacLean CJ *et al.* Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am. J. Hum. Genet*. 71(2), 337–348 (2002).

172 Schwab SG, Knapp M, Mondabon S *et al.* Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. *Am. J. Hum. Genet*. 72(1), 185–190 (2003).

173 Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science* 288(5466), 678–682 (2000).

174 Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* 28(1), 53–67 (2000).

175 Chowdari KV, Mirnics K, Semwal P *et al.* Association and linkage analyses of RGS4 polymorphisms in schizophrenia. *Hum. Mol. Genet*. 11(12), 1373–1380 (2002).

176 Gogos JA, Morgan M, Luine V *et al.* Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc. Natl Acad. Sci. USA* 95(17), 9991–9996 (1998).

177 Egan MF, Goldberg TE, Kolachana BS *et al.* Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc. Natl Acad. Sci. USA* 98(12), 6917–6922 (2001).

178 Shifman S, Bronstein M, Sternfeld M *et al.* A highly significant association between a COMT haplotype and schizophrenia. *Am. J. Hum. Genet*. 71(6), 1296–1302 (2002).

179 Akil M, Kolachana BS, Rothmond DA *et al.* Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. *J. Neurosci*. 23(6), 2008–2013 (2003).

180 Cagnin A, Brooks DJ, Kennedy AM *et al.* *In vivo* measurement of activated microglia in dementia. *Lancet* 358(9280), 461–467 (2001).

181 Klunk WE, Engler H, Nordberg A *et al.* Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Ann. Neurol*. 55(3), 306–319 (2004).

182 Crow TJ. A continuum of psychosis, one human gene and not much else–the case for homogeneity. *Schizophr. Res*. 17(2), 135–145 (1995).

183 Angst J. Historical aspects of the dichotomy between manic-depressive disorders and schizophrenia. *Schizophr. Res*. 57(1), 5–13 (2002).

184 Moller HJ. Bipolar disorder and schizophrenia: distinct illnesses or a continuum? *J. Clin. Psychiatry* 64(Suppl. 6), 23–27 (2003).

185 Ferguson JA, Steemers FJ, Walt DR. High-density fiber-optic DNA random microsphere array. *Anal. Chem*. 72(22), 5618–5624 (2000).

Websites

201 BAC Transgenic Database
www.gensat.org
(Viewed April 2004)

202 Allen Institute for Brain Science
www.brainatlas.org
(Viewed April 2004)

203 Neurome
www.neurome.com
(Viewed April 2004)

204 The International HapMap Project
www.hapmap.org
(Viewed April 2004)

205 NCBI/Genbank
www.ncbi.nih.gov
(Viewed April 2004)

206 NINDS:NIMH Microarray Consortium
http://arrayconsortium.tgen.org
(Viewed April 2004)

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