Systems Approach to Neurodegenerative Disease Biomarker Discovery

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Abstract

Biomarkers are essential for performing early diagnosis, monitoring neurodegenerative disease progression, gauging responses to therapies, and stratifying neurodegenerative diseases into their different subtypes. A wide range of molecular markers are under investigation in tissues and biofluids as well as through imaging; moreover, many are prominent proteins present in cerebrospinal fluid. However, in more frequently and easily collected fluids such as plasma, these proteins show only a modest correlation with disease and thus lack the necessary sensitivity or specificity for clinical use. High-throughput and quantitative proteomic technologies and systems-driven approaches to biofluid analysis are now being utilized in the search for better biomarkers. Biomarker discovery involves many critical steps including study design, sample preparation, protein and peptide separation and identification, and bioinformatics and data integration issues that must be carefully controlled before independent confirmation and validation. In this review, we summarize current proteomic and nucleic acid technologies involved in the discovery of biomarkers of neurodegenerative diseases, particularly Alzheimer's, Parkinson's, Huntington's, and prion diseases.

INTRODUCTION

Neurodegenerative (ND) disease is a large class of complex diseases including Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal dementia (FTD), Huntington's disease (HD), and prion disease. These diseases share one key neuropathological feature: the accumulation of fibrillar aggregates of proteins that result from protein misfolding. Accumulation of misfolded proteins is now considered a key event in the pathogenesis and progression of these ND diseases (1–3). Proteins that are prone to misfold include amyloid-beta (Aβ), associated with AD (4–6); tau, associated with AD and FTD (2, 7–9); α-synuclein, associated with PD (10, 11); and polyglutamine-containing proteins, associated with HD (12). Recent studies of these misfolded proteins suggest that they share key biochemical characteristics and propagation mechanisms with prion protein. As with the pathogenic prion protein, misfolded proteins in other diseases can function as a template to catalyze the misfolding of the normal proteins and their assembly into insoluble, β-sheet-rich, fibrillar aggregates (4).

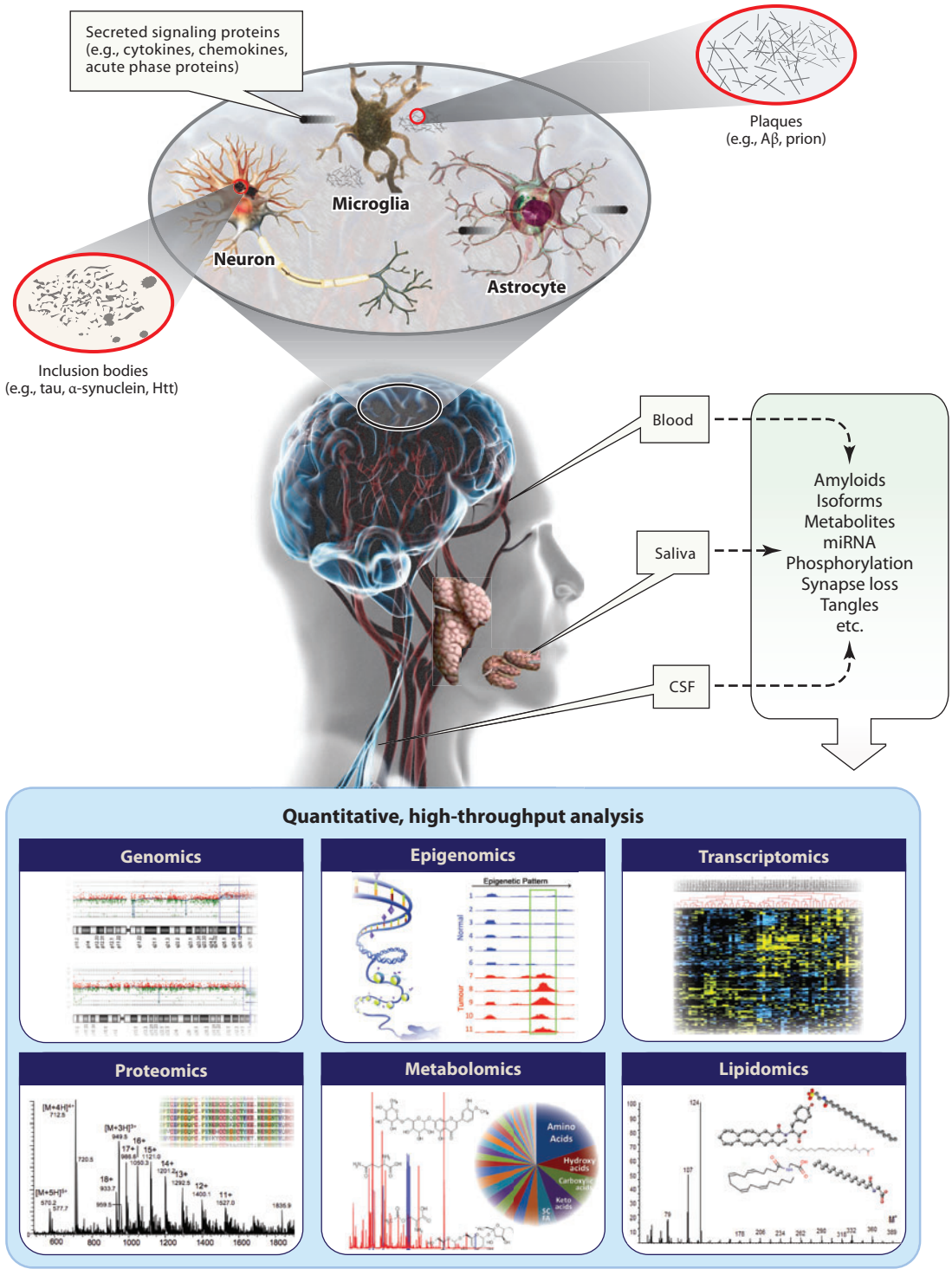
At this time, ND diseases are chronic, progressively debilitating, and incurable. The disabling effects for individuals with ND diseases may continue for years or even decades. The prevalence of ND diseases has increased globally, most noticeably in the aging populations of the developed world. Among different types of ND diseases, AD appears to be the most common: In the United States, 5.4 million people are affected (13); worldwide, more than 33.9 million (14). AD prevalence is forecasted to triple over the next 40 years, which will place a heavy burden on society and its health-care systems in terms of both economic costs and human impacts. Thus, methods for early and reliable detection of various ND diseases are urgently needed to improve patients' quality of life and to provide necessary health and social care. Numerous quantitative, high-throughput technologies have recently been introduced into the search for fluid biomarkers (**Figure 1**). Reliable biomarkers can also facilitate the development of therapeutics to slow the progression of ND diseases.

Neurodegenerative Diseases Are Systemic Disorders

Like most diseases, the ND diseases affect not just the neurological system. The central nervous system (CNS), which includes the brain and the spinal cord, plays an essential role in all aspects of life, including some level of regulation of all other systems in the human body. Alterations in CNS function inevitably lead to systemic dysfunction involving multiple outside organs. For example, PD is classified as a degenerative CNS disorder that affects movement. However, nonmotor symptoms related to olfaction (reduced ability to smell), eye movement (rapid eye movement during sleep), gastrointestinal and urogenital functions, and postural hypotension are common in PD patients. HD is another example; patients with HD often suffer ailments in peripheral tissues including pneumonia, heart conditions, and muscle weakness. Whereas brain dysfunction may contribute to these abnormalities, the disease-causative gene, *huntingtin*, is ubiquitously expressed. Such expression may directly cause various cellular dysfunctions including defective

Figure 1

Pathophysiological hallmarks, targeted biofluids, and sample analysis methodologies for neurodegenerative disease biomarker discovery. Hallmarks include intracellular inclusion body formation, extracellular protein aggregation, and inflammation. Aggregate components and related compounds appear in CSF, blood, and saliva. A range of quantitative and high-throughput (omic) technologies now enable parallel monitoring of the many different classes of compounds (e.g., proteins, nucleic acids, lipids, and metabolites). Abbreviations: Aβ, amyloid-beta; CSF, cerebrospinal fluid; Htt, huntingtin; miRNA, microRNA.



energy metabolism, transcriptional dysregulation, and enhanced apoptotic activities in organs outside the CNS (15).

Some ND diseases may affect the normal function of gastrointestinal tract. Most notably, gastrointestinal dysfunction is common in PD. In some PD patients, the initial symptoms can appear first within the digestive tract rather than within the CNS (16, 17). Other ND diseases with connections to the gastrointestinal tract are prion disease and Whipple's disease. Pathogenic prions are initially located in the small intestine and tonsils and move to the brain and spinal cord in the later stages of disease development (18). Whipple's disease is a disorder caused by infection of *Tropheryma whipplei* and characterized by diarrhea and abdominal pain (19, 20). Additionally, approximately 10–40% of patients also present with clinical neurological dysfunctions including dementia with disorientation and memory loss.

Current Diagnostic Approaches

Despite thorough studies of a large number of candidate biomarkers in each of the major ND diseases—e.g., the cerebrospinal fluid (CSF) levels of tau or Aβ proteins for AD—no fluid biomarker has been fully validated. Because biopsy samples are out of the question for ND disease diagnosis, the current diagnoses are based on the combination of genetic testing, cognitive assessment, psychiatric evaluation, and imaging analysis. For some diseases, the final confirmation arrives only with postmortem pathological or molecular analyses of brain tissue.

The causative mutations or genetic risk factors associated with some ND diseases have been identified in the past several years. These include, for example, the trinucleotide length associated with the *huntingtin* gene for HD and mutations in amyloid precursor protein (APP), presenilin 1, presenilin 2, and apolipoprotein E for AD. These findings provide some genetic diagnostic tests for the diseases; however, it is difficult to predict the disease onset, disease progression, and therapeutic response based on these results. In addition, the results from genetic tests do not cover the entire population of patients with ND diseases.

For imaging-based disease diagnosis, four major technologies are regularly used in assessing neurodegeneration: structural magnetic resonance imaging (sMRI), functional MRI (fMRI), magnetic resonance spectroscopy (MRS), and positron emission tomography (PET). MRI measures brain morphology and can detect changes in the gray matter and white matter during the progression of ND disease. In AD, these changes include the loss of neurons, synapses, dendritic branches, and axons, as well as the expansion of CSF spaces. Neuronal loss correlates with Braak stage and directly relates to impaired cognitive function in AD (21). Because there is a significant anticorrelation between neurofibrillary tangle (NFT) density and neuronal counts, sMRI can be used to assess the progression of AD. The rate of ventricular volume enlargement measured by sMRI can also be used to monitor disease progression or response to treatment with therapeutics that are targeted at NFTs and senile plaque pathologies associated with AD (22).

Tissue oxygen levels can be assessed by fMRI, which reflects the cellular (neuronal) activity in specific brain regions (23). fMRI studies in AD patients have found altered patterns in the medial temporal and parietal lobes that correspond to structural changes detected by sMRI (24). PET with ^{18}F-labeled tracers, such as ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG), can be used to image brain activity and aberrant structures. AMYViD^{TM} (^{18}F-florbetapir), a compound that binds to amyloid plaques, allows the imaging of amyloid plaques directly with PET (25). MRS imaging of *N*-acetylaspartate can be used to monitor the functional status of neuronal mitochondria. Reduced levels of this nervous-system-specific metabolite have been found repeatedly, even in the absence of brain atrophy (26). Reviews of current imaging techniques have been authored by Politis et al. (27) and O'Brien (28).

The combination of fluid biomarkers with functional and structural imaging is expected to improve diagnostic accuracy beyond the individual assays. To date, only a small number of studies have explored this strategy. In one study, the misclassification rate for conversion of mild cognitive impairment (MCI) to AD, based on neuropsychological testing and other clinical data, was 41.3%. The combination of MRI and PET imaging with CSF biomarkers reduced this error rate to 28%. FDG PET appeared to add the greatest prognostic information (29). The combination of CSF biomarkers with MRI or CT analysis of atrophy of the medial temporal lobe has been reported to improve AD detection (30, 31).

One challenge for the diagnosis of ND diseases is their complexity. Often, there is no clear distinction among different ND diseases in their clinical presentations. For example, it is difficult to separate some patients with AD or PD from patients with Lewy body dementia (32, 33). This difficulty limits the ability of the phenotype-based diagnostic approaches, such as cognitive assessment, to provide accurate or detailed classification. Also, obtaining well-characterized human samples for biomarker discovery and conducting meaningful clinical trials during drug development are challenging tasks. However, the development of new high-throughput technologies has led to the discovery of numerous new biomarker candidates for each ND disease.

BIOMARKERS OF NEURODEGENERATIVE DISEASES

Samples

The limited availability of tissue samples from disease sites and the overlap of clinical presentations among different types of ND diseases greatly reduce the ability to achieve early and reliable diagnoses. There is an urgent need for informative biomarkers to assist in the study of ND processes, which may lead to the development of disease treatments. Ideally, biomarkers must fulfill numerous criteria, including accessibility through noninvasive methods, specificity for the pathology, reproducibility of test results, reflectivity of changing pathology, and translatability from laboratory to clinic (34). On the basis of their chemistry, most molecular biomarkers can be grouped into three major subtypes: small-molecule biomarkers, protein-based biomarkers, and RNA-based biomarkers. Among them, the most widely reported candidates are protein-based biomarkers, particularly the aggregation-prone proteins central to ND disease pathology but also proteins associated with oxidative stress and inflammation.

Cerebrospinal Fluid

CSF is currently the most frequently analyzed body fluid in biomarker discovery, due to its proximity to the diseased organ (35–39). Relative to blood and many other body fluids, CSF has low protein and RNA concentrations; therefore, the sample preprocessing is much easier than for plasma. However, this also creates a unique problem for CSF in that any blood contamination during the sample collection process will have a significant impact on the composition of protein and RNA in the CSF (40). A significant issue for CSF is the invasive collection procedure. Lumbar puncture requires a highly trained provider and imposes discomfort and some health risk to the patient. This makes it difficult to use for routine screening and for regular follow-up sampling.

Blood, Plasma, and Serum

All organs in the body are perfused by blood to obtain necessary nutrients and remove metabolic waste from cells. Although some molecules in blood are the product of cell lysis through normal

cell turnover, many circulating biomolecules are deliberately released by cells for specific purposes, as exemplified by the hormones that serve to keep the various body organs operating in concert. Any pathological perturbation would be expected to alter the spectra of various molecules in the blood. Therefore, blood and its various subcomponents—such as peripheral blood mononuclear cells, serum, and plasma—are probably the most commonly used biological materials in general biomarker discovery projects. For example, whole-blood RNA has been used to identify transcripts that may be associated with specific pathological conditions (41–43). To obtain RNA free from proteins and cells, serum or plasma prepared from whole blood is commonly used. The main drawback of using blood or its subcomponents for biomarker discovery is the complexity of biomolecules in the sample, as they are derived from all cell types and organs of the body (44, 45). The concentrations of blood proteins vary across a dynamic range of 10^{10}; accordingly, the 21 most abundant proteins that constitute 99% of the blood protein mass obscure the identification of less abundant proteins unless a preprocessing step to remove those abundant proteins or to select a targeted protein subpopulation such as the glycoproteins is employed (46–50).

Urine

Urine also contains proteins, RNAs, and small molecules and metabolites. Urine is easy to obtain and can be collected frequently. Urine has also been used in biomarker discovery for ND disease. For example, the 8-hydroxydeoxyguanosine (8-OHdG) level in urine correlates well with the stage of PD (51, 52), and the urine level of AD7c, a neuronal thread protein, correlates with the severity of dementia in AD (53).

Saliva

More recently, saliva has gained attention in biomarker discovery for ND diseases because the submandibular gland where saliva is produced expresses both tau and APP, key proteins for various ND diseases (54, 55). The salivary glands are highly vascularized, and the RNAs and proteins in saliva can arise in the glands or move from the blood via diffusion or active transport (56). As with urine, saliva is easy to collect, with or without stimulation. Unstimulated whole saliva is thought to correlate best with systemic clinical conditions, as (chemical) stimulants of saliva production may change the salivary fluid composition.

Animal Models

Although no model can completely mimic the complexity of human diseases, animal models provide valuable contributions to biomarker discovery efforts and are important in linking biomarkers to the molecular mechanisms behind neurodegeneration. In the search for disease markers, animal models can be used to track subtle biochemical, histological, and behavioral changes that occur long before clinical signs appear. Challenges relating to model development include differences in life span between humans and laboratory animals, differences in the fundamental biochemistry or the disease mechanisms between the two, and our limited understanding of the underlying causes of ND disease. Additionally, a majority of highly cited animal research fails to translate to human randomized trials (57), and meta-analysis has revealed statistical significance in animal studies of ND disease that is not replicated in human studies (58). This can be attributed, at least partially, to small-study effects and to publication bias—errors that can be avoided by adopting standards similar to those used in the clinic to ensure that results are based on high-quality and unbiased data.

One approach toward simulating the development of human ND diseases in animals utilizes genetic engineering to overexpress, underexpress, or knock out genes that are significantly associated with the diseases. For example, AD models exhibiting protein accumulation leading to neuronal damage have been created by an overexpression of Aβ or tau and by a transgenic combination of APP, mutated presenilin, and tau. The triple-transgenic mice show a pattern of progression closely matching the human disease. Aβ accumulates first in the cortical regions and then spreads to the hippocampus and amygdala, whereas the tau accumulation moves in the reverse direction (59).

Models of familial PD have been created by targeting PD-related genes such as *α-synuclein* (*PARK1*), *Parkin* (*PARK2*), *UCHL1* (*PARK5*), *PINK1* (*PARK6*), *DJ-1* (*PARK7*), and *LRRK2* (*PARK8*), with varying success. The best of these animal models, which contains the α-synuclein *A53T* mutation, displays much of the desired pathology including α-synuclein aggregation, oligomers, fibrils, phosphorylation, ubiquitination, and progressive neurodegeneration. Only the motor deficits are absent (60). On the basis of reports linking PD to mitochondrial dysfunction, a more complete model has been created by targeting the mitochondrial respiratory pathway in dopaminergic neurons. Cell type–specific inactivation of TFAM (mitochondrial transcription factor A) produces a model with key disease features including late onset, a realistic progression of symptoms, and motor deficits that are reversible with L-DOPA treatment. PD symptoms can also be induced in standard mouse strains through the administration of neurotoxins such as 6-hydroxydopamine and 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (61).

Numerous mouse models have been generated in an effort to emulate the development of human HD through the introduction of a huntingtin gene carrying unstable expansion of CAG repeats—the causative mutation (62). The HD mouse models carrying long CAG repeats recapitulate features of the human disease, with a positive correlation between repeat length and severity of the phenotype. They are being used to study early molecular changes in relevant tissues that start well before overt phenotypic changes appear.

Transgenic mice expressing the human prion gene in the absence of the endogenous mouse prion gene reproduce all of the key features of human prion disease including long asymptomatic periods prior to fatal neurodegeneration (63, 64). Researchers can also experimentally induce standard inbred strains to develop prion disease by inoculating the prion-containing diseased-brain homogenate intracerebrally or intraperitoneally (65, 66). In addition, several lines of prion knockout mice have supported the hypothesis that prion protein is essential for both transmission and pathogenesis of the prion diseases (67, 68). These prion knockout mice have been used as critical controls to filter out biological and systematic noises generated among prion mouse models. It is useful to discuss this in detail as it provides deep insights into the power of the dynamical studies of disease-perturbed networks.

We inoculated the brains of mice with infectious prions and then analyzed the brain transcriptomes of normal and diseased animals at 10 time points across 22 weeks during the progression of the disease, subtracting the transcriptomes of normal mice from those of the diseased mice at each time point. These subtractions identified the differentially expressed genes (DEGs)—approximately 7,400 of the mouse genes were DEGs—which represented an enormous signal-to-noise problem. The noise was of two different types: technical and biological. We developed techniques to help address the technical noise arising from the measurements (66). The approach of adapting multiple inbred-strain/prion-strain mice also allowed us to subtract away much of the biological noise, resulting in approximately 300 DEGs that apparently encoded neurodegeneration induced by pathogenic prion protein. From histopathology studies, we identified four major networks involved in the prion-induced neurodegeneration process: prion replication and accumulation, glial activation, neuronal degeneration, and apoptosis. Approximately 200 of the 300

DEGs mapped into the protein networks that represented these four systems, and the remaining 100 defined six smaller networks that heretofore were not known to be a part of the disease. Three key observations were made from this study. First, the four networks became disease perturbed in sequential order: first prion accumulation, then glial activation, and finally the two neurodegeneration networks. Thus, if one is interested in early detection or effective therapy for the diseases, understanding the initial disease-perturbed network is key. Second, the collective dynamics of these 10 networks explained virtually every aspect of the neuropathology of this disease. Third, if some of the proteins in the disease-perturbed networks are secreted into the blood, they will let us follow the progression of neurodegeneration in the blood. Indeed, some of these proteins were found in the blood (see below). Moreover, the study was instrumental in providing preliminary evidence of the power of using organ-specific (brain-specific) molecules in blood to reflect networks and biological processes that are perturbed in prion disease. These studies allowed us to (*a*) diagnose the prion disease 10 weeks before any clinical signs were apparent and (*b*) follow the progression of the disease by delineating just when each of the networks became disease perturbed. The order and change in the blood reflected the timing of the disease perturbations seen by transcriptome analyses in the brain.

Methods Used in Biomarker Discovery

Depending on the types of biomolecules, several different methods are commonly used in biomarker discovery. For identifying protein-based biomarkers, the traditional method is to use 2-dimensional gel electrophoresis (2DGE) to separate the proteins from samples on the basis of charge and size differences. Distinct protein spots can then be excised from the gel and analyzed by mass spectrometry (MS) to determine the protein identity. This approach is extremely useful in identifying changes associated with posttranslational protein modifications and protein isoforms in the samples (69, 70). However, this process is time consuming, difficult to achieve with consistent reproducibility, challenging for small or hydrophobic proteins, and ambiguous when multiple proteins comigrate to the same spot on the gel. Moreover, 2DGE displays only a small fraction of the total proteins in tissues or blood (71).

With the development of quantitative, high-performance MS in the past decade, its direct use as a discovery tool is now possible (72). Quantitative techniques not only identify proteins on the basis of unique peptide mass/charge ratios but also extrapolate protein concentrations from peptide signal magnitudes. Identification accuracy depends on the completeness of the search database, and detection limits vary with the instruments and techniques used. The numerous instruments and analysis techniques can be grouped into two major approaches for biomarker discovery: labeled and label free. However, thus far, neither labeled nor label-free methods can be used effectively in identifying posttranslational modifications or protein isoform changes in the samples. The labeled approaches, such as isobaric tags for relative and absolute quantitation (iTRAQ) (73) and stable isotope labeling by amino acids in cell culture (SILAC) (74), significantly reduce operation-related sample-to-sample variations because all samples are mixed and processed at the same time after being labeled with different tags. The number of samples that can be used in one experiment depends on the availability of tags—currently, eight independent tags are available for iTRAQ. Compared with the labeled approaches, the label-free approach provides more flexibility in the number of samples that can be included in an experiment and requires less hands-on operation. However, the label-free method requires more computation time for data analysis and higher consistency from sample preparation. It also requires a stable mass spectrometer because it depends on comparing data from independent MS runs. The drawback of either the 2DGE or MS-based protein biomarker discovery approach is the number of proteins that can be

surveyed in a single experiment (usually several hundred for body fluids and two to three thousand for tissue/cell samples). A recent study (75) reported that combining the results from data obtained from targeted proteomics with data obtained from the SWATH™ data acquisition method (AB SCIEX, Foster City, California) could significantly increase the number of proteins that can be measured. This new approach may provide a more comprehensive survey of proteins in various body fluid samples.

Even though the existence of cell-free circulating nucleic acid was reported almost 60 years ago, the recent finding of stable RNA in body fluids makes RNA-based biomarkers a new exciting area. RNA has long been considered only the conduit that links the information (DNA) and the execution (protein) parts of the biological system. This monolithic view of RNA has completely changed in recent years. RNA is involved in every aspect of biological activity, including structural, enzymatic, informational, and regulatory roles (76–79). microRNAs (miRNAs), small regulatory RNAs in cells, constitute probably one of the most investigated extracellular RNAs in body fluids. The levels of certain miRNAs in the circulation correlate well with different pathological conditions; examples include the level of miR-122 for various liver diseases, miR-141 for prostate cancers, and miR-499 and miR-1 for cardiovascular conditions (80–83). Due to the sensitivity of the method and to the limited number of known miRNA species in humans (approximately 800), quantitative polymerase chain reaction (qPCR) is the most commonly used method to conduct surveys on miRNAs in circulation. In addition to miRNAs, the circulation contains other types of RNA, including protein-coding messenger RNAs (mRNAs) and other noncoding RNAs (ncRNAs). Because the RNA concentration in body fluids usually is low, it is not possible to use traditional profiling methods such as microarrays to survey the changes in the spectrum of RNAs in blood. The recent development of highly parallelized next-generation sequencing technologies has provided an opportunity to conduct comprehensive surveys of RNA in the circulation. We have used this approach to identify numerous RNA species including long intervening noncoding RNAs (lincRNAs), mRNAs, and miRNAs, all found in the peripheral circulation. Relevant to biomarker discovery, their concentrations in plasma have a good correlation with liver injury induced by acetaminophen overdose in a mouse model (84). We believe this approach might be applicable to other diseases, including ND disease.

MS is probably the most suitable approach for metabolomic biomarker discovery. The Human Metabolome Database (**http://www.hmdb.ca**) contains more than 7,000 small-molecule metabolites derived from endogenous and exogenous biomolecules that have been detected in the body. One of the biggest challenges in metabolomics is to identify disease-specific metabolites and to pinpoint the origins of the molecules.

Urgent Need for Standard Operating Procedures

Even though many body fluids are readily available and routinely processed in clinical laboratories, varying sample collection procedures (e.g., stimulated versus unstimulated saliva, serum versus plasma, type of anticoagulant used in plasma preparation, method of RNA isolation, quantification platform) often affect the outcome of biomarker discovery and validation. For example, several studies report a difference of RNA and protein concentrations between serum and plasma (85–87). For circulating RNA—especially for the miRNAs, which have short sequence length (from 17 to 22 nucleotides long) and high sequence similarity among the different members—the correlations from profiling results among different measurement platforms have been low (88, 89). Therefore, comparisons of the results from different platforms pose a problem. To facilitate the discovery and validation of biomarkers, it is critical to have standard operating procedures for sample collection, preparation, transportation, and storage as well as for the measurement platforms.

Table 1 Notable fluid protein biomarkers

Disease	Biomarker	Category	Change	Comments
AD	CSF Aβ42	Aggregation	Down	Also down in PD
	CSF Aβ42/Aβ40	Aggregation	Down	
	CSF tau	Aggregation	Up	
	CSF APP isoforms	Aggregation	Weak up	
	CSF BACE1	Aggregation	Up	
	CSF MCP-1, CD14	Inflammation	Weak up	
	CSF SOD1	Oxidative stress	Unchanged	Possible pharmacodynamic marker
	Urine AD7c	Inflammation	Up	
PD	CSF α-synuclein	Aggregation	Down	Also up in CJD
	CSF DJ-1	Oxidative stress	Up	
	Plasma DJ-1	Oxidative stress	Up	
	Serum 8-OHdG	Oxidative stress	Up	Also up in AD and ALS
	Urine 8-OHdG	Oxidative stress	Up	
HD	CSF clusterin	Inflammation	Up	

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; Aβ, amyloid-beta; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BACE1, β-site APP-cleaving enzyme 1; CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; HD, Huntington's disease; MCP-1, monocyte chemoattractant protein 1; PD, Parkinson's disease; SOD1, superoxide dismutase 1.

Protein-Based Biomarkers

Most ND diseases are characterized by specific abnormal modifications, i.e., misfolded protein aggregates. The main components of these intra- and interneuronal insoluble fibrils are extremely promising biomarker candidates that are being extensively investigated and validated (**Table 1**).

Amyloid-beta. Aβ, the major component of extracellular senile plaques in AD, is a by-product of APP metabolism. Abnormal deposition of $A\beta_{1-40}$ and $A\beta_{1-42}$, the main species of Aβ in the brain, is attributed to unusually high levels of Aβ inside brain cells due to either an increased production in familial AD or a decreased clearance in sporadic AD (90, 91). The measurement of Aβ species ($A\beta_{1-40}/A\beta_{1-42}$) in CSF is a reliable diagnostic biomarker of AD (92). However, published results have found no or low correlations between plasma Aβ or CSF Aβ and PET amyloid plaques (93, 94). Due to the universal expression of APP in the body, measurements of plasma Aβ are subject to more sources of variability than are those of plasma CSF Aβ. The complexity of blood-brain barrier and blood-CSF barrier functions induces even greater variation (95). It is believed that further clinical research and assay development will be needed before plasma Aβ can be applied as a state, risk, or trait biomarker of AD.

The key proteins involved in Aβ production, APP and β-site APP-cleaving enzyme 1 (BACE1), are also of great interest as CSF biomarkers. The cleavage of APP by BACE1 produces an amyloidogenic C-terminal fragment (C99) and releases soluble APPβ. C99 is then cleaved by γ-secretase to produce the Aβ peptide. Although reports vary for soluble APP levels, BACE1 levels correlate with Aβ in AD. Of interest, CSF BACE1 levels are even higher in patients with amnestic MCI compared with AD patients (96, 97).

Tau protein. Tau protein is the main component of intraneuronal NFTs observed in AD, FTD, and a group of more than 20 other tau-opathies. This microtubule-associated protein is crucial in axonal functions and highly abundant in neurons of the CNS but less common elsewhere, including in CNS astrocytes and oligodendrocytes. Both of these characteristics, along with its molecular diversity arising from alternative splicing and posttranslational modification (phosphorylation), make tau a promising biomarker of a range of ND diseases.

One property of abnormal hyperphosphorylated tau (p-tau) is high resistance to enzymatic proteolysis, which results in its accumulation in the neurons. As tau hyperphosphorylation can occur in the early stages of disease development, even before the first clinical symptoms appear (Braak stage 1), CSF p-tau could be not only a useful diagnostic and surrogate marker in the advanced stages of the disease but also a prognostic marker in the earliest stages, even before the onset of the disease, i.e., in MCI (98). This possibility has been supported by recent research that showed elevated CSF total tau (t-tau) and p-tau in early AD comparable to those in advanced AD (99). Notably, persistent high levels of CSF t-tau and p-tau over long periods during the disease process are needed to predict the progression of MCI to AD and to distinguish AD/MCI-AD from other tau-opathies and from axonal damage arising from traumatic brain injury, multiple sclerosis, or other ND diseases (100).

α-Synuclein. α-Synuclein is a major component of Lewy bodies and Lewy neurites (the hallmark protein inclusions in PD, dementia with Lewy bodies, and multiple system atrophy) and has been a major area of inquiry for PD treatments and biomarker discovery. Although α-synuclein has long been assumed to be an intracellular protein, recent studies have confirmed the presence of α-synuclein in body fluids such as CSF and blood, both in PD and normal individuals (101). However, thus far, no correlation has been reported between CSF/blood α-synuclein levels and PD disease severity or duration (102–104). In recent years, extracellular α-synuclein oligomers/fibrils, which may derive from dying neurons, have been observed to induce neurotoxic and neuroinflammatory effects in several neuronal and microglial cell culture studies (105, 106). These findings suggest that α-synuclein could be a seeding agent in the initiation and progression of neurodegeneration (107). It would be useful to develop specific and efficient oligomer α-synuclein assays for body fluids to further biomarker research.

Inflammation. A great deal of recent evidence suggests that neuroinflammation is a key process in ND diseases. Activated microglia are regularly found surrounding plaques and secreting proinflammatory cytokines. It has been hypothesized that activated microglia have both neuroprotective and neurotoxic effects, although it is not known at what point chronic inflammation shifts the balance from beneficial to damaging (108, 109). Biomarkers of CNS inflammation are therefore being sought to help observe this shift and discover the pathogenic mechanisms. Additionally, these biomarkers will be necessary for developing therapies that involve microglial activation. Numerous potential markers have been investigated.

Proteins produced by activated macrophages are elevated in the CSF of AD patients. These include chitotriosidase, YKL-40, monocyte chemoattractant protein 1 (MCP-1), and CD14 (110–113). Although the primary functions of the enzymes chitotriosidase and YKL-40 are unknown, these closely related proteins seem to have a role in inflammation and tissue remodeling. The chemokine MCP-1 is a microglial secretion factor that appears to play an important role in macrophage migration. The surface protein CD14 serves as a cofactor for Toll-like receptors (TLRs)—essential components for pathogen recognition in the innate immune system. Microglial TLRs and CD14 are involved in the inflammatory reaction surrounding Aβ deposits (114). Furthermore, increased levels of soluble CD14 have been observed in CSF from PD patients in

addition to that of AD patients (25). Alone, these microglial markers are probably insufficient for AD diagnosis. In each case, disease specificity is insufficient, and independent studies have not always produced consistent findings. Yet these markers may prove useful in combination with other types of biomarkers or in stratifying patients into subgroups.

A proteomic study of HD plasma utilizing 2DGE, MS, and ELISA assays observed several proteins related to the innate immune response, including clusterin and α_2-macroglobulin. Both have links to the pathogenesis of ND disease; clusterin is upregulated in the HD brain, colocalizes with Aβ plaques, and is elevated in AD CSF (115).

Oxidative stress. Attenuated levels of copper-zinc superoxide dismutase 1 (SOD1) have been observed in brain tissue from AD patients (116). Reduced levels of an antioxidant such as SOD1 could change the response to oxidative stress and contribute to neuronal cell loss. As mentioned above, urinary 8-OHdG levels have been shown to change in PD. This product of oxidized DNA increases in a stage-dependent manner. Another protein of great interest in PD is DJ-1, a member of the peptidase C56 family. DJ-1 acts as a sensor for oxidative stress and is believed to protect neurons against it (117). Whereas some PD studies have reported both elevated CSF and plasma DJ-1 levels (118, 119), others have measured no difference in serum DJ-1 concentrations between PD patients and healthy controls (120).

RNA-Based Biomarkers

As for many other diseases, all categories of blood RNA have been analyzed in the search for biomarkers of ND disease. For example, decreased leukocyte gene expression levels of *adrenoceptor alpha 2C* (*ADRA2C*) and *defensin 5* (*DEF5*) (121), *CD243 antigen* (*ABCB1*) (122), and *chemokine* (*C-C motif*) *ligand 5* (*RANTES*) (123) have been reported to distinguish AD patients from controls. Other studies have reported that increased levels of *specificity protein 1 (SP1)* (124), *Toll-like receptor 2* (*TLR2*) and *Toll-like receptor 4* (*TLR4*) (125), and *granulin* (*GRN*) (126) are linked to AD. A set of 96 genes (127) and 170 gene probes (128) also have been used to separate patients from healthy individuals with good sensitivity and specificity.

Decreased levels of *suppression of tumorigenicity 13* (*ST13*), *B-cell CLL/lymphoma 11B* (*BCL11B*) (129), and *leucine-rich repeat containing 8C* (*LRRC8C*) (130) and increased levels of *α-synuclein* (131) have been reported to correlate with PD. Molochnikov et al. (132) identified a panel of five genes that separate early/mild PD patients from healthy age-matched controls with 90.3% sensitivity and 89.1% specificity: *S-phase kinase-associated protein 1 (SKIP1a)*, *huntingtin interacting protein 2* (*HIP2*), *aldehyde dehydrogenase 1 family member A1* (*ALDH1A1*), *proteasome 26S subunit ATPase 4* (*PSMC4*), and *heat shock 70 kDa protein 8* (*HSPA8*). The performance of the five-gene classifier was further validated in an independent set of 30 patients at the advanced stage of PD, classifying correctly all cases as PD (100% sensitivity) (132).

Although circulating RNAs, especially miRNAs, have been studied extensively in diseases such as cancer, their analyses are still relatively new in ND diseases. Several reports describe the involvement of miRNAs in the brain tissues of patients with different ND diseases, such as the downregulation of Let-7i, miR-101, miR-106, miR-107, miR-124a, miR-15, miR-181c, miR-210, miR-22, miR-26b, miR-298, miR-328, miR-34a, miR-363, and miR-93, and the upregulation of miR-125b, miR-128, miR-146, miR-197, miR-320, miR-511, and miR-9 in AD (133, 134). Regarding body fluids, only a few miRNA-related studies address ND diseases (38, 135–137, 138, 139).

Margis et al. (140) identified a set of six blood miRNAs, three of which give good separation between untreated PD patients and healthy individuals (miR-1, miR-22*, and miR-29), and

three of which can distinguish treated from untreated PD patients (miR-16–2*, miR-26a2*, and miR-30a). Khoo et al. (139) compared the miRNA spectra of plasma samples from patients with PD with those of normal individuals and identified numerous miRNAs showing concentration changes in the plasma of PD patients. The results were further validated with a different set of samples, and four miRNAs (miR-1826, miR-450b-3p, miR-626, and miR-505) produced a positive predictive power with 91% sensitivity and 100% specificity.

For AD, Geekiyanage et al. (141) reported a decrease in miR-137, miR-181c, miR-9, miR-29a, and miR-29b levels in serum from patients with probable AD. In addition to miRNAs in blood, those in CSF have also been examined in AD patients as potential biomarkers. Using a qPCR panel, Cogswell et al. (138) reported 60 differentially expressed miRNAs in CSF samples between controls (nondemented, Braak stage 1) and AD patients (demented, Braak stage 5). In another study, Alexandrov et al. (38) identified four miRNAs (miR-146a, miR-155, miR-9, and miR-125b) with higher levels in CSF from AD patients relative to controls.

Using NT2, a neuronally committed human teratocarcinoma cell line that overexpresses mutant huntingtin exon 1, Gaughwin et al. (136) identified a significant induction of miR-34b and miR-1285. The increase of miR-34b levels in plasma was also observed in individuals with premanifest HD compared with normal individuals. These findings support the possibility of developing miRNA-based biomarkers from blood or CSF in different ND diseases.

Lipid and Metabolite Biomarkers

For ND diseases, lipid peroxidation is a common phenomenon. Increased levels of numerous oxidized products such as isoprostanes and isofurans derived from arachidonic acid, and neuroprostanes derived from docosahexaenoic acid can be found in the CSF of patients with AD or HD. However, the quantification of isoprostanes in plasma and urine of AD patients has yielded inconsistent results compared with their quantification in CSF. Xu et al. (142) has reviewed the application of metabolomic techniques to AD biomarker discovery.

FUTURE PROSPECTS

For biomarker discovery, the ability to combine and relate the data between blood and tissue is a powerful approach because it provides the means to assess the extent to which the blood concentrations reflect the disease process in the target organ(s) by following the changes in concentration of specific molecules that are associated with the biological processes in the diseased organs. However, for ND diseases, tissue access through biopsy is usually not possible, and postmortem tissue samples usually do not reflect early-stage pathology. A good animal model might be one approach to compensate for the deficiency of human samples and thus might help elucidate the networks associated with ND diseases. Recent advances in generating induced pluripotent stem cells from patients may also provide a unique opportunity to understand the etiology and underlying mechanism of ND diseases. Another key approach will be to use the blood as a window into the normal and diseased biology of the brain, and a systems approach to understanding the molecular spectrum in blood may facilitate this (**Figure 2**).

Systems Biology

The challenge in identifying biomarkers is to distinguish a small signal from an immense amount of noise. For example, the typical approach to blood-based biomarker discovery is to compare the molecular profiles of blood samples from normal individuals with those from diseased individuals;

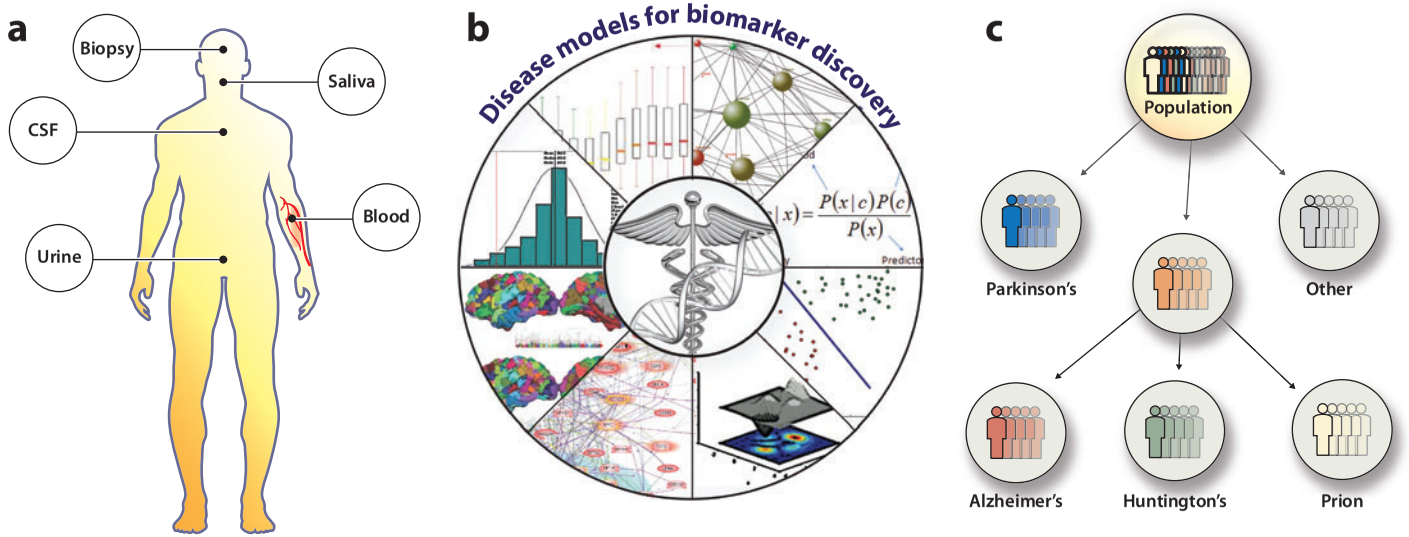


Figure 2

The systems approach can provide novel opportunities for developing neurodegenerative disease diagnostics. (*a*) Biopsy samples provide the clearest and most direct molecular indicators of neurodegenerative disease. However, potentially useful diagnostic biomarkers can also be found in cerebrospinal fluid (CSF), blood, saliva, and urine. This emphasizes the need for comprehensive screening of these other fluids. After assessment by high-throughput screening, some biofluid biomolecules can serve as proxies for the presence of disease. (*b*) Models based on qualitative and quantitative systems biology methods can facilitate the discovery of novel biomarkers of neurodegenerative disease. Panels of biomarkers and diagnostic models can be used to predict the onset of neurodegeneration, indicate disease class (and subtype), and perhaps even elucidate distinct pathological processes. (*c*) Once robust models have been established, patient populations can be accurately stratified into disease groups. Importantly, systems approaches can be applied to the identification of patient groups that share common therapeutic regimens (hopefully at earlier stages of disease) so that the appropriate clinical intervention can be provided only to patients likely to respond.

inevitably, enormous numbers of differences are found. However, 99% of these differences are noise, so the question is, How does one distinguish real signals from noise? Indeed, most published biomarker discovery studies in the past have failed to provide reliable biomarkers because of their inability to distinguish signal from noise (143). Fortunately, a systems approach to biomarkers provides a multiplicity of powerful tools for identifying such signals.

Using systems biology to understand disease mechanisms and identify informative biomarkers. A systems approach recognizes biology and medicine as information sciences that involve two fundamental types of information: the digital genome and the environmental signals that come from outside the genome. Biological networks connect these two types of information and shape their resulting phenotypes, be they normal or diseased. In a diseased state, one or more biological networks is perturbed genetically and/or environmentally, and these perturbations alter the patterns of gene expression within the diseased organ. These dynamically changing and expanding altered patterns of gene and protein expression can reflect the recruitment of additional disease-perturbed networks, indicate the progression of the disease, and explain the fundamental pathogenesis of the disease.

A systems approach to understanding the etiology and mechanism of diseases employs two strategies: (*a*) assessing the changes in the informational molecules (e.g., ncRNAs, mRNAs, or proteins) in the diseased tissues across the progression of the disease, and (*b*) understanding how the dynamics of the inferred networks correlate with the etiology and progression of the disease. However, because access to tissue samples through biopsy is usually impossible for ND diseases, most of the mechanistic studies for various ND diseases have been conducted with postmortem tissues or animal models. One significant drawback of postmortem tissues is that they reflect only the final stage of the disease; therefore, it is difficult to identify the etiology and the associated networks at the disease's early stages. A good example of the systems approach using an animal model to study both early and late stages is the inducible prion disease application described above.

A systems approach to the discovery of blood (and tissue) biomarkers. A systems approach to the discovery of blood (and tissue) biomarkers should utilize the following principles:

- Blood is the ideal fluid for accessing biomarkers because it bathes all organs and contains secreted/released proteins from each of these organs. Changes in biomarker concentrations in the blood can thus reflect changes in the disease state of their cognate organs.
- The diagnostic analyses should be carried out in a longitudinal manner so that changes in disease state can be followed.
- The analyses should be quantitative, as concentration changes are important diagnostic features.
- In humans, each patient should be his/her own control.
- The analyses should measure multiple markers because testing the status of multiple networks within the organ of interest is advantageous, and probably necessary.
- Biomarkers may be of different informational types: mRNAs, miRNAs, proteins, metabolites, or lipids. Preliminary evidence suggests that blood proteins and circulating miRNAs are going to be extremely effective biomarkers (34, 80, 144).

The discovery phase of biomarker development should employ systems approaches to deal with the signal-to-noise problem outlined above—and these are of many diverse types. For example, the use of organ-specific blood proteins is powerful because each has the protein address of its origin. Also, researchers have identified brain cell type–specific transcripts (145), some of which

THE POWER OF MULTIPARAMETER BIOMARKER PANELS

We illustrate the approach to multiparameter panels by describing a recent study on a 13-protein blood panel that has the ability to distinguish benign lung nodules from their cancerous counterparts (147). In the United States, three million lung nodules are discovered each year through imaging, and the question is what to do with them. One can choose to wait, to use PET imaging or needle aspiration, or to perform bronchoscopy or surgical removal. Approximately 20% of the patients are subject to expensive procedures, and almost half of these are carried out on benign nodules. We created the panel to minimize false positives by using high-throughput, quantitative MS and by targeting 190 selected proteins through the following strategy:

1. Compile a large list of potential biomarker candidates from secreted and membrane proteins that are differentially expressed in normal versus cancer tissue cultures. Also include candidates from the literature.
2. Limit the list to proteins known to have been identified in blood.
3. Assess levels in numerous normal and disease plasma samples. Limit the list to proteins that are reliably detectable.

We analyzed these 190 proteins against plasmas from 72 individuals with benign lung nodules and 72 with neoplastic lung nodules. On the basis of their individual classification ability, we took the top-scoring 36 proteins and randomly assigned them to one million panels of 10 proteins. We rank-ordered the panels according to their classification ability, then asked which were the "cooperative" proteins seen most frequently in the highest-ranking panels. With this approach, we identified 13 "cooperative" proteins.

To validate the 13-protein panel, we collected independent samples from 52 normal and 52 neoplastic nodules from different individuals at four different sites. This 13-protein panel had the ability to identify 90% of the neoplastic nodules and, more important, the ability to identify more than 40% of the benign nodules and thus rule them out as neoplastic. In addition, 12 out of 13 proteins mapped into three key disease-perturbed networks in lung cancer and thus gave one the ability to follow the progression (or reemergence) of lung cancer. This nicely illustrates how a systems approach to blood biomarker discovery can produce a powerful diagnostic panel. The same approach can be applied to any of the neurodegenerative diseases.

might be secreted into the blood and hence might serve as useful reflections of cell type–specific disease. Combining these methods with the use of induced pluripotent stem cells derived from patients leads to the possibility of using biomarkers to pinpoint cell type–specific pathologies. This approach also allows evaluation of how secreted molecules from normal-tissue culture cells differ from those from diseased-tissue culture cells of the same type. There are many additional systems approaches for the identification of candidate disease biomarkers.

These candidate biomarkers must be taken through both a discovery phase and a validation phase. Indeed, the Institute of Medicine report on developing omics-based biomarkers offers excellent criteria for the selection of disease biomarkers (143). (See also sidebar, The Power of Multiparameter Biomarker Panels.)

The advantages of organ-specific biomarker fingerprints. Currently, biomarkers are identified by comparing the general molecular profile (e.g., proteins in the blood or other body fluids) between healthy and diseased individuals. A fundamental limitation to this approach is the signal-to-noise problem mentioned above. Moreover, if there is a change in a biomarker's concentration in a body fluid, one cannot be certain which organ in the body induced the changes because most

molecules in body fluid originate from multiple organs. Each organ appears to release into the blood multiple molecules, including proteins, mRNAs, and miRNAs, some of which are organ specific. Collectively, these represent an organ-specific fingerprint in the blood. If the organ is healthy, the levels of these molecules in the organ-specific fingerprint will be set at a "steady" level (or range) for each molecule (and for each individual). If the organ is diseased, the blood levels of molecules whose cognate biological networks have become perturbed will be affected. Because each disease perturbs different combinations of networks, one can use these organ-specific fingerprints to distinguish health from disease and determine which disease. If one has on the order of 50 organ-specific blood biomarkers from each of the 50 major organs and evaluates them every year, they will provide a wellness assessment that will be effective in visualizing transitions from health to disease in any of these organs.

Tools for Measurements

We can use either next-generation DNA sequencing or DNA arrays to analyze global changes of mRNAs and miRNAs. For protein, we can use targeted proteomics to analyze approximately 100 proteins across a dynamic range of approximately 10^5 with a midattomole level of sensitivity (146). In the future, we will be able to analyze many proteins from microfluidic chips. Today it is possible to analyze approximately 30 proteins from a fraction of a droplet of blood in about 5 minutes on a microfluidic chip with ELISA assays (J. Heath, personal communication). When more effective protein-capture agents are available, we can imagine chips that will let us measure 2,500 proteins in the same manner (50 organ-specific proteins from each of 50 organs), and this will open blood protein analyses to the billions of samples that we will need to analyze in the future.

Data Analysis Challenges for Harnessing Blood as a Window to Neurodegeneration

The identification of high-accuracy biomarkers to track ND diseases in the blood will likely require combining sophisticated computational approaches with high-sensitivity measurements. The challenges associated with harnessing the rich molecular milieu of the blood will necessitate a range of systems approaches to overcome. For example, changes in blood measurements of any data type can be induced not just by a particular ND disease, but by a whole host of environmental, genetic, and disease factors—making the distinction of a relevant signal from noise highly challenging. Computational analysis for making blood an effective window thus introduces a hierarchy to the disease diagnosis problem, whereby increasingly specific questions need to be answered: Is a disease present? Where is it located? What should the diagnosis and subsequent therapy be? This challenge can be met, in part, through the use of coarse-to-fine hierarchies that move from broader to more specific questions and through the corresponding adjustment of the biomarkers' specific properties at each level. For example, the first-pass biomarkers need not be highly specific for disease but could be general warning signs that come, e.g., from enhanced immune response. The second layer could be built from organ-specific proteins that would help determine that the dominant disease perturbation is located in the brain. Subsequent layers of the classification hierarchy would be targeted more toward molecules that are related to the disease pathology and that are most specifically perturbed in the particular disease at increasingly fine spatial or tissue-type resolution.

In such a scenario, it is unlikely that any one data type will provide enough sensitivity and specificity to fully differentiate disease states; therefore, multiple-omic approaches will be needed.

The blood contains a plethora of different sources of information (as detailed above) that can be algorithmically harnessed. One question concerning multiple-omic approaches is whether diagnostics based on the different omics can lead to combinations of molecular diagnostics with error rates that have relatively low correlation with one another. If so, multiple omics tests could be combined to greatly improve overall diagnostic accuracy.

Additionally, even with advanced machine learning and large-scale omics data, it is hard to imagine that statistical approaches alone would provide us with sufficient statistical power to learn all that needs to be learned. Thus, harnessing mechanistic context and a rich understanding of the underlying biological phenomena will also be of crucial importance in focusing the search space and amplifying the signal. Such approaches can leverage the increasing power of biological networks as they become better mapped. Taken together, such computational approaches operating on exponentially increasing amounts of high-throughput data hold substantial promise for the use of systems medicine to better treat disease and maintain wellness.

Conclusion

Because the brain is difficult to biopsy, the identification of blood biomarkers that reflect disease in the brain is critical. The new systems approach to blood diagnostics holds significant promise for uncovering blood biomarkers that accurately reflect normal and diseased brains. Because these panels of biomarkers will probably reflect the dynamics of disease-perturbed networks, there is hope that these panels will provide early diagnosis, follow the progression of disease, follow the response to therapy, and stratify the disease into its different subtypes. We believe that the power of making blood a window into health and disease will usher in a new era of systems medicine (148) that will make health care more effective, reduce the cost of health care, and promote innovation in dealing with both disease and wellness.

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LITERATURE CITED

1. Frost B, Diamond MI. 2010. Prion-like mechanisms in neurodegenerative diseases. *Nat. Rev. Neurosci.* 11(3):155–59
2. de Calignon A, Polydoro M, Suárez-Calvet M, William C, Adamowicz DH, et al. 2012. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* 73(4):685–97. Erratum. 2012. *Neuron* 76(2):461
3. Hardy J, Revesz T. 2012. The spread of neurodegenerative disease. *N. Engl. J. Med.* 366:2126–28

4. Soto C. 2012. Transmissible proteins: expanding the prion heresy. *Cell* 149(5):968–77
5. Morales R, Duran-Aniotz C, Castilla J, Estrada LD, Soto C. 2012. De novo induction of amyloid-β deposition in vivo. *Mol. Psychiatry* 17(12):1347–53
6. Telling GC. 2013. The importance of prions. *PLoS Pathog.* 9(1):e1003090
7. Wu JW, Herman M, Liu L, Simoes S, Acker CM, et al. 2013. Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J. Biol. Chem.* 288(3):1856–70
8. Neumann M, Tolnay M, Mackenzie IR. 2009. The molecular basis of frontotemporal dementia. *Expert Rev. Mol. Med.* 11:e23
9. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, et al. 2005. Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 309(5733):476–81
10. Luk KC, Song C, O'Brien P, Stieber A, Branch JR, et al. 2009. Exogenous α-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proc. Natl. Acad. Sci. USA* 106(47):20051–56
11. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, et al. 2012. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 338(6109):949–53
12. Ren PH, Lauckner JE, Kachirskaia I, Heuser JE, Melki R, Kopito RR. 2009. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat. Cell Biol.* 11(2):219–25
13. Alzheimer's Assoc. 2012. 2012 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 8(2):131–68
14. Barnes DE, Yaffe K. 2011. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.* 10(9):819–28
15. Sassone J, Colciago C, Cislaghi G, Silani V, Ciammola A. 2009. Huntington's disease: the current state of research with peripheral tissues. *Exp. Neurol.* 219(2):385–97
16. Pfeiffera RF. 2003. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol.* 2(2):107–16
17. Hardoff R, Sula M, Tamir A, Soil A, Front A, et al. 2001. Gastric emptying time and gastric motility in patients with Parkinson's disease. *Mov. Disord.* 16(6):1041–47
18. Stack MJ, Vidal-Diez A, Arnold ME, Jones EM, Spencer YI, et al. 2011. Experimental bovine spongiform encephalopathy: detection of PrPSc in the small intestine relative to exposure dose and age. *J. Comp. Pathol.* 145(2–3):289–301
19. Schneider T, Moos V, Loddenkemper C, Marth T, Fenollar F, Raoult D. 2008. Whipple's disease: new aspects of pathogenesis and treatment. *Lancet Infect. Dis.* 8(3):179–90
20. Marth T. 2009. New insights into Whipple's disease—a rare intestinal inflammatory disorder. *Dig. Dis.* 27(4):494–501
21. Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, et al. 1997. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann. Neurol.* 41(1):17–24
22. Silbert LC, Quinn JF, Moore MM, Corbridge E, Ball MJ, et al. 2003. Changes in premorbid brain volume predict Alzheimer's disease pathology. *Neurology* 61(4):487–92
23. Logothetis NK. 2002. The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. *Philos. Trans. R. Soc. B* 357(1424):1003–37
24. Bokde ALW, Lopez-Bayo P, Born C, Ewers M, Meindl T, et al. 2010. Alzheimer disease: functional abnormalities in the dorsal visual pathway. *Radiology* 254(1):219–26
25. Newberg AB, Arnold SE, Wintering N, Rovner BW, Alavi A. 2012. Initial clinical comparison of ^{18}F- florbetapir and ^{18}F-FDG PET in patients with Alzheimer disease and controls. *J. Nucl. Med.* 53(6):902–7
26. Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA. 2007. *N*-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Prog. Neurobiol.* 81(2):89–131
27. Politis M, Su P, Piccini P. 2012. Imaging of microglia in patients with neurodegenerative disorders. *Front. Pharmacol.* 3:96
28. O'Brien JT. 2007. Role of imaging techniques in the diagnosis of dementia. *Br. J. Radiol.* 80(Spec. Issue 2):S71–77
29. Shaffer JL, Petrella JR, Sheldon FC, Choudhury KR, Calhoun VD, et al. 2013. Predicting cognitive decline in subjects at risk for Alzheimer disease by using combined cerebrospinal fluid, MR imaging, and PET biomarkers. *Radiology* 266(2):583–91

30. Zhang Y, Londos E, Minthon L, Wattmo C, Liu H, et al. 2008. Usefulness of computed tomography linear measurements in diagnosing Alzheimer's disease. *Acta Radiol.* 49(1):91–97
31. Schoonenboom NS, van der Flier WM, Blankenstein MA, Bouwman FH, Van Kamp GJ, et al. 2008. CSF and MRI markers independently contribute to the diagnosis of Alzheimer's disease. *Neurobiol. Aging* 29(5):669–75
32. Park KW, Kim HS, Cheon SM, Cha JK, Kim SH, Kim JW. 2011. Dementia with Lewy bodies versus Alzheimer's disease and Parkinson's disease dementia: a comparison of cognitive profiles. *J. Clin. Neurol.* 7(1):19–24
33. Tröster AI. 2008. Neuropsychological characteristics of dementia with Lewy bodies and Parkinson's disease with dementia: differentiation, early detection, and implications for "mild cognitive impairment" and biomarkers. *Neuropsychol. Rev.* 18(1):103–19
34. Etheridge A, Lee I, Hood L, Galas D, Wang K. 2011. Extracellular microRNA: a new source of biomarkers. *Mutat. Res.* 717(1–2):85–90
35. Irwin DJ, Trojanowski JQ, Grossman M. 2013. Cerebrospinal fluid biomarkers for differentiation of frontotemporal lobar degeneration from Alzheimer's disease. *Front. Aging Neurosci.* 5:6
36. Constantinescu R, Mondello S. 2012. Cerebrospinal fluid biomarker candidates for Parkinsonian disorders. *Front. Neurol.* 3:187
37. Choi YS, Choe LH, Lee KH. 2010. Recent cerebrospinal fluid biomarker studies of Alzheimer's disease. *Expert Rev. Proteomics* 7(6):919–29
38. Alexandrov PN, Dua P, Hill JM, Bhattacharjee S, Zhao Y, Lukiw WJ. 2012. microRNA (miRNA) speciation in Alzheimer's disease (AD) cerebrospinal fluid (CSF) and extracellular fluid (ECF). *Int. J. Biochem. Mol. Biol.* 3(4):365–73
39. Romeo MJ, Espina V, Lowenthal M, Espina BH, Petricoin EF III, Liotta LA. 2005. CSF proteome: a protein repository for potential biomarker identification. *Expert Rev. Proteomics* 2(1):57–70
40. Zhang J. 2007. Proteomics of human cerebrospinal fluid—the good, the bad, and the ugly. *Proteomics Clin. Appl.* 1(8):805–19
41. Välk K, Vooder T, Kolde R, Reintam MA, Petzold C, et al. 2010. Gene expression profiles of non-small cell lung cancer: survival prediction and new biomarkers. *Oncology* 79(3–4):283–92
42. Hollander Z, Lin D, Chen V, Ng R, Wilson-McManus J, et al. 2010. Whole blood biomarkers of acute cardiac allograft rejection: double-crossing the biopsy. *Transplantation* 90(12):1388–93
43. Bai VU, Hwang O, Divine GW, Barrack ER, Menon M, et al. 2012. Averaged differential expression for the discovery of biomarkers in the blood of patients with prostate cancer. *PLoS ONE* 7(4):e34875
44. Guipaud O. 2013. Serum and plasma proteomics and its possible use as detector and predictor of radiation diseases. *Adv. Exp. Med. Biol.* 990:61–86
45. Lottspeich F, Kellermann J, Keidel EM. 2010. Molecular biology tools: proteomics techniques in biomarker discovery. *Scand. J. Clin. Lab. Investig. Suppl.* 242:19–22
46. Fanayan S, Hincapie M, Hancock WS. 2012. Using lectins to harvest the plasma/serum glycoproteome. *Electrophoresis* 33(12):1746–54
47. Lee JY, Kim JY, Park GW, Cheon MH, Kwon KH, et al. 2011. Targeted mass spectrometric approach for biomarker discovery and validation with nonglycosylated tryptic peptides from N-linked glycoproteins in human plasma. *Mol. Cell. Proteomics* 10(12):M111.009290
48. Mortezai N, Harder S, Schnabel C, Moors E, Gauly M, et al. 2010. Tandem affinity depletion: a combination of affinity fractionation and immunoaffinity depletion allows the detection of low-abundance components in the complex proteomes of body fluids. *J. Proteome Res.* 9(12):6126–34
49. Tan SH, Mohamedali A, Kapur A, Baker MS. 2013. Ultradepletion of human plasma using chicken antibodies: a proof of concept study. *J. Proteome Res.* 12(6):2399–413
50. Polaskova V, Kapur A, Khan A, Molloy MP, Baker MS. 2010. High-abundance protein depletion: comparison of methods for human plasma biomarker discovery. *Electrophoresis* 31(3):471–82
51. Kikuchi A, Takeda A, Onodera H, Kimpara T, Hisanaga K, et al. 2002. Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiol. Dis.* 9(2):244–48
52. Sato S, Mizuno Y, Hattori N. 2005. Urinary 8-hydroxydeoxyguanosine levels as a biomarker for progression of Parkinson disease. *Neurology* 64(6):1081–83

53. de la Monte SM, Wands JR. 2002. The AD7c-NTP neuronal thread protein biomarker for detecting Alzheimer's disease. *Front. Biosci.* 7:d989–96
54. Conrad C, Vianna C, Freeman M, Davies P. 2002. A polymorphic gene nested within an intron of the *tau* gene: implications for Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 99(11):7751–56
55. Oh YS, Turner RJ. 2006. Effect of γ-secretase inhibitors on muscarinic receptor-mediated calcium signaling in human salivary epithelial cells. *Am. J. Physiol. Cell Physiol.* 291(1):C76–82
56. Chiappin S, Antonelli G, Gatti R, De Palo EF. 2007. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. *Clin. Chim. Acta* 383(1–2):30–40
57. Hackam DG, Redelmeier DA. 2006. Translation of research evidence from animals to humans. *JAMA* 296(14):1731–32
58. Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, et al. 2013. Evaluation of excess significance bias in animal studies of neurological diseases. *PLoS Biol.* 11(7):e1001609
59. Mineur YS, McLoughlin D, Crusio WE, Sluyter F. 2005. Genetic mouse models of Alzheimer's disease. *Neural Plast.* 12(4):299–310
60. Lee MK, Stirling W, Xu Y, Xu X, Qui D, et al. 2002. Human α-synuclein-harboring familial Parkinson's disease-linked Ala-53 → Thr mutation causes neurodegenerative disease with α-synuclein aggregation in transgenic mice. *Proc. Natl. Acad. Sci. USA* 99(13):8968–73
61. Harvey BK, Wang Y, Hoffer BJ. 2008. Transgenic rodent models of Parkinson's disease. *Acta Neurochir. Suppl.* 101:89–92
62. Huntington's Dis. Collab. Res. Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72(6):971–83
63. Collinge J, Palmer MS, Sidle KC, Hill AF, Gowland I, et al. 1995. Unaltered susceptibility to BSE in transgenic mice expressing human prion protein. *Nature* 378(6559):779–83. Erratum. 1997. *Nature* 389(6650):526
64. Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, et al. 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* 83(1):79–90
65. Stephenson DA, Chiotti K, Ebeling C, Groth D, DeArmond SJ, et al. 2000. Quantitative trait loci affecting prion incubation time in mice. *Genomics* 69(1):47–53
66. Hwang D, Lee IY, Yoo H, Gehlenborg N, Cho JH, et al. 2009. A systems approach to prion disease. *Mol. Syst. Biol.* 5:252
67. Prusiner SB, Groth D, Serban A, Koehler R, Foster D, et al. 1993. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc. Natl. Acad. Sci. USA* 90(22):10608–12
68. Büeler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, et al. 1993. Mice devoid of PrP are resistant to scrapie. *Cell* 73(7):1339–47
69. Hu S, Jiang J, Wong DT. 2010. Proteomic analysis of saliva: 2D gel electrophoresis, LC-MS/MS, and Western blotting. *Methods Mol. Biol.* 666:31–41
70. Baumann M, Meri S. 2004. Techniques for studying protein heterogeneity and post-translational modifications. *Expert Rev. Proteomics* 1(2):207–17
71. Gygi SP, Corthals GL, Zhang Y, Rochon Y, Aebersold R. 2000. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. *Proc. Natl. Acad. Sci. USA* 97(17):9390–95
72. Surinova S, Schiess R, Huttenhain R, Cerciello F, Wollscheid B, Aebersold R. 2011. On the development of plasma protein biomarkers. *J. Proteome Res.* 10(1):5–16
73. Evans C, Noirel J, Ow SY, Salim M, Pereira-Medrano AG, et al. 2012. An insight into iTRAQ: Where do we stand now? *Anal. Bioanal. Chem.* 404(4):1011–27
74. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, et al. 2002. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol. Cell. Proteomics* 1(5):376–86
75. Gillet LC, Navarro P, Tate S, Röst H, Selevsek N, et al. 2012. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol. Cell. Proteomics* 11(6):O111.016717

76. Xu Y, Komiyama M. 2012. Structure, function and targeting of human telomere RNA. *Methods* 57(1): 100–5
77. Scott WG, Martick M, Chi YI. 2009. Structure and function of regulatory RNA elements: ribozymes that regulate gene expression. *Biochim. Biophys. Acta* 1789(9–10):634–41
78. Werner A, Sayer JA. 2009. Naturally occurring antisense RNA: function and mechanisms of action. *Curr. Opin. Nephrol. Hypertens.* 18(4):343–49
79. Montange RK, Batey RT. 2008. Riboswitches: emerging themes in RNA structure and function. *Annu. Rev. Biophys.* 37:117–33
80. Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, et al. 2009. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc. Natl. Acad. Sci. USA* 106(11):4402–7
81. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. 2008. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. USA* 105(30):10513–18
82. Gidlöf O, Andersson P, van der Pals J, Götberg M, Erlinge D. 2011. Cardiospecific microRNA plasma levels correlate with troponin and cardiac function in patients with ST elevation myocardial infarction, are selectively dependent on renal elimination, and can be detected in urine samples. *Cardiology* 118(4):217–26
83. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, et al. 2011. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. *Circ. Cardiovasc. Genet.* 4(4):446–54
84. Wang K, Yuan Y, Li H, Cho JH, Huang D, et al. 2013. The spectrum of circulating RNA: a window into systems toxicology. *Toxicol. Sci.* 132(2):478–92
85. Wang K, Yuan Y, Cho JH, McClarty S, Baxter D, Galas DJ. 2012. Comparing the microRNA spectrum between serum and plasma. *PLoS ONE* 7(7):e41561
86. Ishikawa M, Tajima Y, Murayama M, Senoo Y, Maekawa K, Saito Y. 2013. Plasma and serum from nonfasting men and women differ in their lipidomic profiles. *Biol. Pharm. Bull.* 36(4):682–85
87. Frank EA, Shubha MC, D'Souza CJ. 2012. Blood glucose determination: plasma or serum? *J. Clin. Lab. Anal.* 26(5):317–20
88. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, et al. 2010. The microRNA spectrum in 12 body fluids. *Clin. Chem.* 56(11):1733–41
89. Callari M, Dugo M, Musella V, Marchesi E, Chiorino G, et al. 2012. Comparison of microarray platforms for measuring differential microRNA expression in paired normal/cancer colon tissues. *PLoS ONE* 7(9):e45105
90. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, et al. 1999. Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature* 402(6761):537–40
91. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, et al. 2010. Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science* 330(6012):1774
92. Toledo JB, Brettschneider J, Grossman M, Arnold SE, Hu WT, et al. 2012. CSF biomarkers cutoffs: the importance of coincident neuropathological diseases. *Acta Neuropathol.* 124(1):23–35
93. Lui JK, Laws SM, Li QX, Villemagne VL, Ames D, et al. 2010. Plasma amyloid-β as a biomarker in Alzheimer's disease: the AIBL study of aging. *J. Alzheimer's Dis.* 20(4):1233–42
94. Huang Y, Potter R, Sigurdson W, Kasten T, Connors R, et al. 2012. β-Amyloid dynamics in human plasma. *Arch Neurol.* 69(12):1591–97
95. Banks WA, Robinson SM, Verma S, Morley JE. 2003. Efflux of human and mouse amyloid β proteins 1–40 and 1–42 from brain: impairment in a mouse model of Alzheimer's disease. *Neuroscience* 121(2):487–92
96. Zetterberg H, Andreasson U, Hansson O, Wu G, Sankaranarayanan S, et al. 2008. Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease. *Arch. Neurol.* 65(8):1102–7
97. Hampel H, Shen Y. 2009. Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a biological candidate marker of Alzheimer's disease. *Scand. J. Clin. Lab. Investig.* 69(1):8–12
98. Schraen-Maschke S, Sergeant N, Dhaenens CM, Bombois S, Deramecourt V, et al. 2008. Tau as a biomarker of neurodegenerative diseases. *Biomark. Med.* 2(4):363–84
99. Andreasen N, Sjogren M, Blennow K. 2003. CSF markers for Alzheimer's disease: total tau, phospho-tau and Aβ42. *World J. Biol. Psychiatry* 4(4):147–55

100. Ost M, Nylén K, Csajbok L, Ohrfelt AO, Tullberg M, et al. 2006. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. *Neurology* 67(9):1600–4
101. El-Agnaf OM, Salem SA, Paleologou KE, Cooper LJ, Fullwood NJ, et al. 2003. α-Synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J.* 17(13):1945–47
102. Tokuda T, Salem SA, Allsop D, Mizuno T, Nakagawa M, et al. 2006. Decreased α-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. *Biochem. Biophys. Res. Commun.* 349(1):162–66
103. Mollenhauer B, Locascio JJ, Schulz-Schaeffer W, Sixel-Doring F, Trenkwalder C, Schlossmacher MG. 2011. α-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study. *Lancet Neurol.* 10(3):230–40
104. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, et al. 2010. DJ-1 and α-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. *Brain* 133(Pt. 3):713–26
105. Danzer KM, Ruf WP, Putcha P, Joyner D, Hashimoto T, et al. 2011. Heat-shock protein 70 modulates toxic extracellular α-synuclein oligomers and rescues trans-synaptic toxicity. *FASEB J.* 25(1):326–36
106. Zhang W, Wang T, Pei Z, Miller DS, Wu X, et al. 2005. Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J.* 19(6):533–42
107. Danzer KM, Krebs SK, Wolff M, Birk G, Hengerer B. 2009. Seeding induced by α-synuclein oligomers provides evidence for spreading of α-synuclein pathology. *J. Neurochem.* 111(1):192–203
108. Esiri MM. 2007. The interplay between inflammation and neurodegeneration in CNS disease. *J. Neuroimmunol.* 184(1–2):4–16
109. Cameron B, Landreth GE. 2010. Inflammation, microglia, and Alzheimer's disease. *Neurobiol. Dis.* 37(3):503–9
110. Mattsson N, Tabatabaei S, Johansson P, Hansson O, Andreasson U, et al. 2011. Cerebrospinal fluid microglial markers in Alzheimer's disease: elevated chitotriosidase activity but lack of diagnostic utility. *Neuromol. Med.* 13(2):151–59
111. Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, et al. 2010. YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol. Psychiatry* 68(10):903–12
112. Corrêa JD, Starling D, Teixeira AL, Caramelli P, Silva TA. 2011. Chemokines in CSF of Alzheimer's disease patients. *Arq. Neuropsiquiatr.* 69(3):455–59
113. Yin GN, Jeon H, Lee S, Lee HW, Cho JY, Suk K. 2009. Role of soluble CD14 in cerebrospinal fluid as a regulator of glial functions. *J. Neurosci. Res.* 87(11):2578–90
114. Landreth GE, Reed-Geaghan EG. 2009. Toll-like receptors in Alzheimer's disease. *Curr. Top. Microbiol. Immunol.* 336:137–53
115. Dalrymple A, Wild EJ, Joubert R, Sathasivam K, Björkqvist M, et al. 2007. Proteomic profiling of plasma in Huntington's disease reveals neuroinflammatory activation and biomarker candidates. *J. Proteome Res.* 6(7):2833–40
116. Gulesserian T, Seidl R, Hardmeier R, Cairns N, Lubec G. 2001. Superoxide dismutase SOD1, encoded on chromosome 21, but not SOD2 is overexpressed in brains of patients with Down syndrome. *J. Investig. Med.* 49(1):41–46
117. Hardy J, Lewis P, Revesz T, Lees A, Paisan-Ruiz C. 2009. The genetics of Parkinson's syndromes: a critical review. *Curr. Opin. Genet. Dev.* 19(3):254–65
118. Waragai M, Wei J, Fujita M, Nakai M, Ho GJ, et al. 2006. Increased level of DJ-1 in the cerebrospinal fluids of sporadic Parkinson's disease. *Biochem. Biophys. Res. Commun.* 345(3):967–72
119. Waragai M, Nakai M, Wei J, Fujita M, Mizuno H, et al. 2007. Plasma levels of DJ-1 as a possible marker for progression of sporadic Parkinson's disease. *Neurosci. Lett.* 425(1):18–22
120. Maita C, Tsuji S, Yabe I, Hamada S, Ogata A, et al. 2008. Secretion of DJ-1 into the serum of patients with Parkinson's disease. *Neurosci. Lett.* 431(1):86–89
121. Kálmán J, Kitajka K, Pákáski M, Zvara A, Juhász A, et al. 2005. Gene expression profile analysis of lymphocytes from Alzheimer's patients. *Psychiatr. Genet.* 15(1):1–6
122. Chen KD, Chang PT, Ping YH, Lee HC, Yeh CW, Wang PN. 2011. Gene expression profiling of peripheral blood leukocytes identifies and validates ABCB1 as a novel biomarker for Alzheimer's disease. *Neurobiol. Dis.* 43(3):698–705

123. Kester MI, van der Flier WM, Visser A, Blankenstein MA, Scheltens P, Oudejans CB. 2011. Decreased mRNA expression of CCL5 [RANTES] in Alzheimer's disease blood samples. *Clin. Chem. Lab. Med.* 50(1):61–65
124. Villa C, Ridolfi E, Fenoglio C, Ghezzi L, Vimercati R, et al. 2013. Expression of the transcription factor Sp1 and its regulatory hsa-miR-29b in peripheral blood mononuclear cells from patients with Alzheimer's disease. *J. Alzheimer's Dis.* 35(3):487–94
125. Zhang W, Wang LZ, Yu JT, Chi ZF, Tan L. 2012. Increased expressions of TLR2 and TLR4 on peripheral blood mononuclear cells from patients with Alzheimer's disease. *J. Neurol. Sci.* 315(1–2):67–71
126. Coppola G, Karydas A, Rademakers R, Wang Q, Baker M, et al. 2008. Gene expression study on peripheral blood identifies progranulin mutations. *Ann. Neurol.* 64(1):92–96
127. Rye PD, Booij BB, Grave G, Lindahl T, Kristiansen L, et al. 2011. A novel blood test for the early detection of Alzheimer's disease. *J. Alzheimer's Dis.* 23(1):121–29
128. Fehlbaum-Beurdeley P, Sol O, Désiré L, Touchon J, Dantoine T, et al. 2012. Validation of AclarusDx™, a blood-based transcriptomic signature for the diagnosis of Alzheimer's disease. *J. Alzheimer's Dis.* 32(1):169–81
129. Scherzer CR, Eklund AC, Morse LJ, Liao Z, Locascio JJ, et al. 2007. Molecular markers of early Parkinson's disease based on gene expression in blood. *Proc. Natl. Acad. Sci. USA* 104(3):955–60
130. Soreq L, Bergman H, Israel Z, Soreq H. 2012. Exon arrays reveal alternative splicing aberrations in Parkinson's disease leukocytes. *Neurodegener. Dis.* 10(1–4):203–6
131. Mutez E, Leprêtre F, Le Rhun E, Larvor L, Duflot A, et al. 2011. SNCA locus duplication carriers: from genetics to Parkinson disease phenotypes. *Hum. Mutat.* 32(4):E2079–90
132. Molochnikov L, Rabey JM, Dobronevsky E, Bonucelli U, Ceravolo R, et al. 2012. A molecular signature in blood identifies early Parkinson's disease. *Mol. Neurodegener.* 7:26
133. Delay C, Mandemakers W, Hébert SS. 2012. MicroRNAs in Alzheimer's disease. *Neurobiol. Dis.* 46(2):285–90
134. Hébert SS, De Strooper B. 2009. Alterations of the microRNA network cause neurodegenerative disease. *Trends Neurosci.* 32(4):199–206
135. Sheinerman KS, Tsivinsky VG, Crawford F, Mullan MJ, Abdullah L, Umansky SR. 2012. Plasma microRNA biomarkers for detection of mild cognitive impairment. *Aging* 4(9):590–605
136. Gaughwin PM, Ciesla M, Lahiri N, Tabrizi SJ, Brundin P, Björkqvist M. 2011. Hsa-miR-34b is a plasma-stable microRNA that is elevated in pre-manifest Huntington's disease. *Hum. Mol. Genet.* 20(11):2225–37
137. Ai J, Sun LH, Che H, Zhang R, Zhang TZ, et al. 2013. MicroRNA-195 protects against dementia induced by chronic brain hypoperfusion via its anti-amyloidogenic effect in rats. *J. Neurosci.* 33(9):3989–4001
138. Cogswell JP, Ward J, Taylor IA, Waters M, Shi Y, et al. 2008. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. *J. Alzheimer's Dis.* 14(1):27–41
139. Khoo SK, Petillo D, Kang UJ, Resau JH, Berryhill B, et al. 2012. Plasma-based circulating microRNA biomarkers for Parkinson's disease. *J. Parkinson's Dis.* 2:321–31
140. Margis R, Margis R, Rieder CR. 2011. Identification of blood microRNAs associated to Parkinson's disease. *J. Biotechnol.* 152(3):96–101
141. Geekiyanage H, Jicha GA, Nelson PT, Chan C. 2012. Blood serum miRNA: non-invasive biomarkers for Alzheimer's disease. *Exp. Neurol.* 235(2):491–96
142. Xu XH, Huang Y, Wang G, Chen SD. 2012. Metabolomics: a novel approach to identify potential diagnostic biomarkers and pathogenesis in Alzheimer's disease. *Neurosci. Bull.* 28(5):641–48
143. Inst. Med. Natl. Acad. 2012. *Evolution of Translational Omics: Lessons Learned and the Path Forward.* Washington, DC: Natl. Acad. Press
144. Qin S, Zhou Y, Lok AS, Tsodikov A, Yan X, et al. 2012. SRM targeted proteomics in search for biomarkers of HCV-induced progression of fibrosis to cirrhosis in HALT-C patients. *Proteomics* 12(8):1244–52
145. Ko Y, Ament SA, Eddy JA, Caballero J, Earls JC, et al. 2013. Cell type-specific genes show striking and distinct patterns of spatial expression in the mouse brain. *Proc. Natl. Acad. Sci. USA* 110(8):3095–100

146. Hüttenhain R, Surinova S, Ossola R, Sun Z, Campbell D, et al. 2013. N-glycoprotein SRMAtlas: a resource of mass spectrometric assays for N-glycosites enabling consistent and multiplexed protein quantification for clinical applications. *Mol. Cell. Proteomics* 12(4):1005–16
147. Li XJ, Hayward C, Fong PY, Dominguez M, Hunsucker SW, et al. 2013. A blood-based proteomic classifier for the molecular characterization of pulmonary nodules. *Sci. Transl. Med.* 5(207):207ra142
148. Hood L, Flores M. 2012. A personal view on systems medicine and the emergence of proactive P4 medicine: predictive, preventive, personalized and participatory. *N. Biotechnol.* 29(6):613–24

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