Published in final edited form as:

Med Chem. 2016 ; 12(3): 217–225.

miRNAs as Circulating Biomarkers for Alzheimer's Disease and Parkinson's Disease

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Abstract

Detection of markers for neurodegenerative disorders (NDDs) within brain tissue of Alzheimer's disease (AD) or Parkinson's disease (PD) patients has always been hampered by our inability to access tissue from living human subjects and obtain biopsy samples of key regions implicated in disease occurrence and progression. Currently, diagnosis of NDDs is principally based on clinical observation of symptoms that present at later stages of disease progression, followed by additional neuroimaging and, possibly, CSF evaluation. A way to potentially detect and diagnose NDDs at a far earlier stage is to screen for abnormal levels of specific disease markers within the peripheral circulation of patients with NDDs. Increasing evidence suggests that there is dysregulation of microRNAs (miRNAs) in NDDs. Peripheral blood mononuclear cells as well as biofluids, such as plasma, serum, urine and cerebrospinal fluid, contain miRNAs that can be identified and quantified. This opens the potential for circulating levels of miRNAs within blood or other biofluids to be characterized and used as a non-invasive diagnostic biomarker screen to support early disease detection and possible disease progression monitoring of NDDs such as AD and PD. Plainly, such a potential screen is only possible with a clear understanding of which miRNAs change with disease, and when this occurs during the progression of AD and PD. Such information is becoming increasingly available and in the near future may not only support disease diagnosis but provide the opportunity to evaluate therapeutic interventions earlier in the disease process where their targets may be more relevant to delay AD or PD progression.

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Keywords

Alzheimer's disease; β -amyloid; α -synuclein; biofluids; diagnostic biomarkers; miRNAs; Parkinson's disease

Introduction

All cells, whether neuronal or non-neuronal, possess multiple adaptive homeostatic regulatory mechanisms to allow them to rapidly cope with continuously changing physiological demands, to survive acute periods of intense stress and adapt to milder but chronic stress. Different stresses encompass misfolded/damaged proteins, high biosynthetic or secretory demands, changes in redox balance (oxidative/nitrosative stress), inflammation and aging – together with environmental and genetic insults. Depending on the magnitude of these and the vulnerability of neurons and their synapses, pathways that lead towards pruning or, more severely, neuronal death can be triggered. Contingent on the neuronal population/brain area involved, these actions can lead to one of several types of neurodegenerative disorders (NDDs) via initial sub-apoptotic events, such as synapse loss to impact neuronal function, that ultimately progress into neuronal loss. There are several limitations, such as our lack of ability to perform a biopsy on neural tissues in humans, which make it problematic to reliably detect markers for neurodegenerative disorders (NDDs) in neurological disorders epitomized by Alzheimer's disease (AD) and Parkinson's disease (PD). Presently, NDDs are primarily diagnosed based on clinical observations of symptoms and neuroimaging techniques. Recent studies have revealed different mechanisms contributing to the etiology of NDDs. Dysregulation of microRNAs (miRNAs) has been proposed as one such mechanism that contributes towards the pathogenesis of NDDs [1]. In fact, the process of neurodegeneration can be considered as a "RNA disorder" [2,3]. MiRNAs comprise of small non-coding, single stranded RNA molecules (~22 nucleotides). They impact gene expression through base pairing to the messenger RNA (mRNA) and initiating the process of translation repression [4].

Recent research has shown that miRNAs play a pivotal role in myriads of physiological processes by modifying and/or modulating the expression of thousands of genes, and thereby affect gene regulation [5,6,7,8,9]. MiRNA deregulation has been implicated in a growing number of NDDs [10]. Autopsy brain findings and *in vivo* animal studies have revealed that nearly 70% of the known miRNAs are expressed within the central nervous system with a distinct localization [11]. MiRNAs are transported in the blood in liposomes, high density lipoproteins and other proteins; thus protecting them from degradation [12,13]. In addition, peripheral biofluids, including plasma, serum and cerebrospinal fluid, in addition to peripheral blood mononuclear cells (PBMCs) contain microRNAs (miRNAs) that can be readily identified and quantified [14,15,16,17]. This would imply that levels of circulating miRNAs in blood or serum of individuals may potentially serve as non-invasive and economically affordable biomarkers for the early diagnosis of NDDs, such as AD and PD. If true, then miRNAs sampled from blood or other biofluids have the potential to be used for screening, early detection and disease progression monitoring of NDDs.

miRNAs as Biomarkers in AD

AD is a disorder that primarily impacts the world's elderly, with a global prevalence of close to 50% among individuals aged 85 and older. Patients with AD eventually suffer total disability and succumb to an earlier death. The number suffering from AD is nearly 30 million people worldwide, and this figure is projected to rise in the coming decades [18]. AD is a NDD entailing specific pathological characteristics that involves the key elements of progressive loss of memory and cognitive abilities due to neuronal degeneration, beta-amyloid (β -amyloid) plaque accumulation and neurofibrillary tangle formation [19,20]. Impairments and loss of synaptic functions are to be the consequence of abnormal generation and accumulation of β -amyloid oligomers and deposits within the brain [21,22]. β -amyloid, particularly in soluble oligomeric form, is associated with generation of superoxide free radicals that can ultimately induce vascular endothelial damage, neuronal deterioration and neuroinflammation [23,24].

In addition to later events in AD, typified by β -amyloid plaque deposition and formation of neurofibrillary tangles, there are earlier events in AD pathogenesis such as deregulation of miRNA that are equally important, possibly more important, in the early diagnosis of AD [25]. Since the expression of disease-coding genes may be regulated by specific miRNAs, it has been proposed that alterations in miRNA expression may result in the accrual of disease-causing proteins and ensuing neuronal degeneration that culminates in AD [26]. Various studies have investigated the changes in the expression of miRNA in AD in an effort to elucidate the underlying pathogenic mechanisms of AD progression [27]. In this regard, studies have revealed that marked changes in miRNA expression are noted in various brain regions when the postmortem human brains of AD patients are profiled [28]. Certain miRNAs have been shown to be linked with deregulation of defined genes implicated in the pathogenesis of AD [29]. For instance, miR-146, miR-106, miR-9, miR-29, miR-107, miR-181, miR-128, miR-125b, miR-210 and miR-34 are among those miRNAs that have been identified to be dysregulated in AD and, hence, these may serve as potential diagnostic biomarkers for AD, as will be discussed herein.

Amyloid precursor protein (APP), a membrane protein playing a crucial role in neural plasticity and regulation of synapses and, additionally, the protein from which β -amyloid is cleaved, has been reported to be a target for miR-106 dysregulation in AD. Studies on anterior temporal cortex of AD subjects have provided support for direct binding of miR106a and miR106b to APP mRNA and the subsequent downregulation of these miRNAs [30,31]. Such studies thereby support the potential utility of miR-106 as a potential biomarker for AD. Similarly, a rapid lowering of miR-9 levels is observed upon the experimental addition of β -amyloid to primary neuron maintained in culture, which not only provides a correlation of plaque formation with miR-9 deregulation [32] but also highlights miR-9 as a possible diagnostic biomarker for AD disease progression. Likewise, fluorescent miRNA array analysis of the hippocampal brain region of fetal, control adult and AD patients discovered the occurrence of heightened levels of miR-128, miR-125b and miR-9 within the AD subjects [33]. In a separate study, downregulation of miR-210 and miR-9 and upregulation of miR-125b have been described in AD brains, hence suggesting that deregulated brain miRNAs have the potential to serve as biomarkers for AD pathogenesis

pathways that impact processes that include neurogenesis, β -amyloid processing and insulin resistance [34].

In a more comprehensive study, Burgos *et al.* used next generation small RNA sequencing to profile the content of miRNAs from 69 AD patients, 67 PD patients and 78 neurologically healthy controls [35]. miRNA content was analyzed in serum as well as cerebrospinal fluid obtained postmortem from subjects on which a full neuropathology evaluation was also conducted. In this study, 41 miRNAs were found to be significantly dysregulated within the CSF of AD patients, as compared to age-mated health controls, whereas 20 miRNAs were differentially expressed within the serum samples of AD patients versus controls. This study notably reported the presence of 13 novel miRNAs in addition to the evaluated pathological changes, which included the expected amyloid plaque deposition and neurofibrillary tangles within the brain of AD subjects and the presence of Lewy body pathology in the brain of PD subjects. Most of the novel miRNAs have also been reported by other researchers as being dysregulated in AD and/or PD. This study crucially demonstrated an association between the presence of dysregulated miRNAs in peripheral cell-free CSF as well as serum and severity of neurodegenerative pathological changes in the brains of AD and PD patients. Likewise, in a separate study, fluorescent miRNA-array-based analysis of CSF from 6 AD patients and 6 healthy subjects reported a significant elevation (increases ranged from 1.4-fold to 3-fold) in the levels of miR-9, miR-34a, miR-125b, mmir-155, miR-28 and miR-146a in AD patients versus controls [36]. The same study also demonstrated that these miRNAs were sensitive to the proinflammatory protein nuclear factor-kappa-B, to thereby implicate their role in the progressive inflammatory-induced neuronal degeneration that occurs in the process that ultimately results in AD.

When miRNA expression was studied in peripheral blood mononuclear cells in a study performed on 16 AD patients and 16 normal elderly controls, miR-34a and miR-181b were found to be significantly upregulated in peripheral blood mononuclear cells of AD patients compared to healthy controls [37]. In one clinical study carried out on human brain tissues with different degrees of AD-type pathologies that were compared to healthy controls, miR-107 was found to accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE 1, also known as β -secretase activity). Diminished levels of miR-107 were strongly correlated with elevated BACE 1 mRNA levels with an increasing severity of AD-type pathology within the samples [38], implicating miR-107 as a potential diagnostic biomarker for AD worthy of further evaluation. In this regard, miR-146a is a further notable diagnostic potential biomarker of AD progression consequent to the fundamental role that miR-146a plays in the interrelationship between AD and inflammatory processes via a progressive upregulation of neuroinflammatory gene expression. In accord with this, upregulation of miR-146a, in particular, has been described in the hippocampus and temporal cortex of the brain of AD patients [39,40]. The relevance of this finding is supported by a more recent study in which miRNAs were identified in the CSF of AD patients and age-matched non-demented control subjects using quantitative polymerase chain reaction, levels of miR-146a were found to be significantly lower in CSF of the AD group [41].

Deregulated miRNAs in blood or plasma can be used as potential biomarkers in the diagnosis of AD. When whole blood samples from 94 AD patients and 21 healthy controls were subjected to next-generation sequencing and subsequent miRNA target enrichment analysis, a 12-miRNA signature was characterized in the AD group (has-let-7d-3p, let-7f-5p, miR-26a-5p, miR-26b-5p, miR-103a-3p, miR-107, miR-5020-3p, miR-112, miR-161, miR-151a-3p, miR-1285-5p, miR-532-5p) that could be used to differentiate between AD and controls with a notable 93% accuracy and 95% specificity [42]. Along a similar line, another study explored the possibility of using pairs of brain-enriched human plasma miRNAs as biomarkers to differentiate among AD patients, mild cognitive impairment (MCI) patients and cognitively normal subjects. Findings of this study indicated two sets of miRNA pairs (miR-323-3p/-370, miR-134/-370 and miR-382/-370) and (miR-132/-491-5p, miR-128/-491-5p and miR-874/-491-5p) that usefully distinguished AD and MCI patients from control subjects. However, this set of miRNA pairs was found to be common within AD and MCI patients [43], making further differentiation between AD and MCI more complex.

In addition to the profiling of miRNA's in NDDs, such as AD, there is also a need to appraise the temporality of these events. Specifically, the question as to whether or not there are any differentially expressed miRNAs present during the progression of a disorder, i.e., from the prodromal phase of AD to full blown disease, is important to evaluate and answer? Amyloid- β deposition, in the case of AD, as a prime example of a key pathological feature, appears to occur well before any cognitive decline is apparent. In light of this one could then ask whether or not the levels of select miRNAs that associate with amyloid- β follow a time-dependence? Could any of these miRNA then be used as risk factor markers and can therapies that reverse such trends possibly help in preventing or delaying disease onset or progression? In this regard, for miRNAs that provide insight into mechanistic processes that drive AD pathology (whether amyloid- β or phosphorylated tau deposition, or loss of a brain or CSF marker that is tied to a physiological function altered during disease) mitigation of their changes from the values of healthy aged-matched controls might provide insight into ameliorative strategies. As an example, serine palmitoyltransferase (SPT) is the first rate-limiting enzyme in the *de novo* synthesis of ceramide. Ceramides, composed of sphingosine and a fatty acid, are present in high levels within neuronal cell membranes, particularly in lipid rafts, and have a variety of physiological functions that include pro-apoptotic ones. There is substantial evidence for dysregulation of SPT levels having a role in AD, and notably that SPT may directly impact amyloid- β levels. In this regard, Geekiyanage and Chan demonstrated that ceramides as well as SPT levels were elevated in the brain cortices of a subgroup of sporadic AD patients. It was also shown that two long SPT subunits, specifically SPT long chain 1 and SPT long chain 2, are post-transcriptionally modulated by miR-137 / miR-181c and miR-9 / miR-29a/b, respectively. In addition, significant correlation was noted between SPT, their corresponding miRNAs and the deposition of β -amyloid in autopsy brain samples of AD patients [44]. This is further supported by an earlier study by Herbert et al. that showed that miR-29a/b plays an important role in amyloid- β production [30]. More importantly, in a separate later study, Geekiyanage *et al.* confirmed that the circulating levels of miR-137, miR-9, miR-29a/b and miR-181c are, in fact, low in the blood serum of probable AD patients, as compared to healthy controls. In addition, these

miRNA levels were also found to be noticeably lower in the serum of AD risk factor models [45].

Using a global profiling approach, Kumar et al. measured a total of 654 circulating miRNAs from a total of 11 AD and 20 healthy human subjects' plasma, and found a distinct circulating 7-miRNA signature (hsa-let-7g-5p, hsa-let-7d-5p, hsa-miR-142-3p, hsa-miR-15b-5p, hsa-miR-545-3p, hsa-miR-191-5p, and hsa-miR-301a-3p) in AD samples. These miRNAs were significantly down-regulated [46]. More importantly, this unique circulating 7-miRNA signature in plasma was further validated using an independent cohort of 20 AD patients and 17 healthy control subjects. The results of this validation revealed positive correlations (with 95% prediction accuracies) across the two independent cohorts, and established that AD patients can be distinguished from healthy, control subjects with a high accuracy.

miRNAs as Biomarkers in PD

Parkinson's disease is the second most common neurodegenerative disorder afflicting 1% – 3% of the elderly population above 65 years of age, with global prevalence of 4.1 to 4.6 million people [47,48]. PD leads to gradual decline of numerous brain functions, particularly motor function but also cognition, and results in early demise [49]. Classically, clinically manifesting in the form of slow movement of voluntary muscles, resting tremor, muscle stiffness and eventual instability of balance and posture, the disorder is characterized by death and loss of dopaminergic neurons (DNs) of the substantia nigra, a brain region critical in regulating body movement via its projection areas [50,51]. Due to significant heterogeneity of the disease itself, PD symptoms show person to person variability [52, 53], and increasing evidence suggest neurotransmitters in addition to the dopaminergic system are compromised, including the noradrenergic, serotonergic and cholinergic systems that underpin non-motor symptoms. Currently existing PD treatments only provide symptomatic relief, with medicines primarily impacting dopaminergic function or acting as anti-inflammatory agents.

miRNAs have been reported to be involved in the pathogenesis of PD [54,55]. Hence, miRNAs may serve as potential biomarkers for the diagnosis of PD. A key feature of the disease is the abnormal accumulation of α -synuclein protein aggregates in the form of insoluble fibrils within the presynaptic terminals in PD brain [56]. Of note, following sequence analysis, it has been reported that human the α -synuclein gene is highly conserved throughout the entire 3'-untranslated region, which suggests a role of miRNA regulation [57]. In particular, α -synuclein has been found to be the target of two miRNAs, namely, miR-7 and miR-153. These two miRNAs appear to function synergistically to downregulate the mRNA and protein levels of α -synuclein by binding to the 3'-untranslated region of α -synuclein [58]. This observation is supported by findings from *in vitro* studies in which miR-7 has been described to suppress α -synuclein-induced cytotoxicity in neuronal cultures [59]. Likewise, an analysis of brain tissue samples from PD patients defined select miRNAs that appear to be significantly elevated. As an example, miRNAs altered in PD brain include miR-21*, miR-26b, miR-224, miR-373*, miR-301b and miR-106b that specifically target components of the chaperone-mediated autophagy pathway [60], thereby contributing to

Lewy body pathology [61]. Further analysis of miRNAs that regulate critical physiological functions associated the onset of disease pathology likely will provide additional miRNAs worth evaluating as potential diagnostic biomarkers for PD.

Particularly attractive as biomarkers of a disease process are those that may be present in biological fluids of PD patients versus healthy control individuals. As an example, peripheral blood samples from 8 untreated PD patients were compared with 8 healthy, control subjects, and the differential presence of three miRNAs (miR-1, miR-22* and miR-29a) was noted in the PD patients following quantitative reverse transcription polymerase chain reaction analysis [62]. As a further example, a separate clinical study evaluated plasma samples from 25 healthy controls versus 31 untreated PD patients. Seven miRNAs (miR-137, miR-193a-3p, miR-125a-3p, miR-196b, miR-454miR-181c, and miR-331-5p) were found over-expressed in the plasma from PD patients; thereby highlighting these as worthy of further analysis as potential PD biomarkers [63].

In an interesting study investigating the involvement of miRNAs in the etiology of PD, miRNA expression profiling was performed on PBMCs obtained from 19 PD patients and 13 healthy controls using microarrays. The results determined that 18 miRNAs were differentially expressed in PD patients as compared to the controls. Furthermore, chromatin immunoprecipitation-sequencing analysis was performed to unmask genome-wide interactions of α -synuclein, and the merging of this data with miRNomics data as well as *in silico* analysis of the genes involved in PD revealed that the glycosphingolipid biosynthesis pathway as well as the protein ubiquitination pathway were key contributors to PD disease progression. Three miRNAs were reported as the prime regulators of these two biologically important pathways, specifically miR-26a, miR-30b and miR-30c, and two genes (ST8SIA4 and USP37) were, in particular, linked with PD pathogenesis [64].

Soreq *et al.* undertook an interesting evaluation of miRNAs obtained from blood leukocytes of 7 PD patients before and after treatment by electrical stimulus-induced deep brain stimulation, and compared the results to those of 6 healthy control subjects following a comprehensive miRNA profiling by next-generation small-RNA sequencing and exon and splice junction microarrays [65]. Sixteen miRNAs were differentially expressed in PD patients, as compared to the control subjects, and the expression pattern of 11 of these miRNAs were modified by deep brain stimulation. Of these 11 miRNAs altered by electrical stimulation, 5 (specifically, miR-1249, miR-20a, miR-18b*, miR-378c, miR-4293) matched those present in the healthy control group; signifying a reversal of the PD miRNA pattern to that of healthy individuals following stimulation.

Occasionally, the absence of or abnormally low levels of certain miRNAs may infer the onset of a NDD. The optimal functioning of dopaminergic neurons appears to importantly depend on a well-designed miRNA network, based on evidence obtained from both cell culture as well as *in vivo* studies. Investigations on drosophila in addition to mouse models have demonstrated that disruption of key miRNA networks in midbrain DNs lead to a progressive loss of neurons, and thereby causes impaired motor activity [52]. miR-133b is specifically expressed in the midbrain DN of healthy individuals but, interestingly, miR-133b is completely absent in the midbrain tissues obtained from PD patients. It has

been suggested that miR-133b regulates the maturation and function of midbrain DNs via a negative-feedback circuit, which includes paired-like homeodomain transcription factor Pitx3 [66]. PITX3 can regulate the expression of neurotrophic factors (Brain derived neurotrophic factor and glial cell derived neurotrophic factor) that are critical for the dopaminergic neuron growth and survival, and polymorphisms in the PITX3 gene associate with sporadic and early-onset PD. Hence, monitoring for potential declines miR-133b levels in the elderly or those at risk for PD may help aid in the identification of individuals that may be in the process to results in PD as, like Alzheimer's and other chronic NDDs, a long clinically silent process occurs before symptom manifestation – and this may allow therapeutic measures may be undertaken when disease targets are more relevant to potentially curtail the onset of this disorder.

Recent common-variant association studies have discovered that a simple mutation within the Leucine-rich repeat kinase 2 (LRRK2) gene can trigger sporadic PD [67,68]. This finding is reinforced by investigations in PD mouse model that have shown that the overexpression of the LRRK2 gene instigated enhanced α -synuclein-induced neurodegeneration. In contrast, the inhibition of LRRK2 gene expression improved or halted neuronal degeneration caused by α -synuclein [69]. On investigation of the levels of LRRK2 protein and its targeted miRNAs in the frontal cerebral cortex of patients with sporadic PD (16 PD patients versus 7 healthy control subjects), LRRK2 protein expression levels were found to be markedly increased but levels of miR-205 expression were decreased in the brain of those with sporadic PD [70]. Likewise, studies on primary neuron cultures and neuronal cell lines have demonstrated that miR-205 regulates the expression of LRRK2 protein. Together, these studies suggest that downregulation of miR-205 may underwrite abnormally high levels of LRRK2 protein within the brain of sporadic PD patients, and support further evaluation of miR-205 as a potential biomarker for the early diagnosis of sporadic PD – particularly for individuals with ethnic backgrounds (Ashkenazi Jewish and North African Arab Berbers) where mutations in LRRK2 account for a far greater number of PD cases than in the general population.

Current Challenges and Future of miRNA Biomarker Discovery

Currently, the diagnosis of AD and PD is primarily based on the clinical observation of symptoms and is supported by neuroimaging analysis to exclude the likelihood of other neuropsychiatric disorders [71]. As a consequence the development of AD and PD are generally only recognized at a late stage during their disease course, when the window of opportunity for intervention is limited [72, 73]. Although neuroimaging techniques incrementally aid in the diagnosis of NDDs [71], these techniques have been shown not to have adequate diagnostic accuracy to clearly differentiate between AD (or PD) from a healthy subject at an individual level [74], as among factors several pathological features, such as amyloid- β deposition, can occur in elderly cognitively normal brain [75]. Although positron emission tomography imaging can potentially differentiate cases of AD from normal non-AD cases, a meta-analysis of the operating characteristics of PET, performed on studies conducted between 1989 and 2003, revealed limitations of PET imaging with extensive variations in sensitivity and specificity [76]. Certainly, there have been improvements over the past decade in computerized image processing methods to allow, for

example, the comparison of a subject's images to a group of control images. This can provide important advantages for detecting significant metabolic changes with greater objectivity across centers, but the detection of prodromal AD still in large part relies on subtle functional brain changes [77]. An additional way to potentially detect and diagnose NDDs at much early stage is by analyzing abnormal levels of specific disease markers, such as deregulated miRNAs within the peripheral circulation of subjects – particularly in familial or at high risk populations for developing NDDs – that can compliment existing diagnostic techniques.

Clearly, like any other technologies, there are limitations and the detection/quantification of miRNAs as potential diagnostic biomarkers is no exception. Although the majority of extracellular RNA extraction methods generate pure and high quality RNA, it has been shown that a different RNA yield is achieved from diverse RNA methods in relation to the RNA size profile as well as quantity [78]. It has been suggested that this difference may be due to the fact that current vesicular isolation methods produce a mixture of vesicles that differ in their lipid membrane composition and RNA content; thus causing dissimilarity in their susceptibility to lysis [79]. In addition, variations in isolated RNA species appear to be quite pronounced depending on whether RNA is isolated from CSF, plasma, serum or some other tissue. As an example, Burgos et al. compared different RNA extraction methods employed directly on CSF and plasma, and significant differences in RNA yield were observed [80]. Furthermore, highly sensitive techniques, such as miRNA sequencing, display high variability in profiling especially when working with human tissue samples obtained from various sources. In this regard, tissues might derive from human patients potentially on medications that could impact miRNA profiling or from subjects with comorbid conditions or other unrelated and undiagnosed acute or chronic disorders. When miRNA profiles undergo changes consequent to medications, it can be difficult to differentiate between the drug treatment actions on miRNA profiles and the effect of the disease on miRNA expression [81].

Further current challenges encountered in handling studies dealing with large datasets and/or a large number of human subjects are information reproducibility and availability. In the field of microarrays that has developed a long history of use, the data is often incompletely annotated and, as a result, the analyses are difficult to reproduce. Hence, the developing area of small RNA-based biomarker development is still facing the issue of replication of published results by independent scientists [82], and further work is required.

When the effect sizes associated with biomarkers reported in highly cited individual articles (from more than 400 citations) and in consequent meta-analyses were compared, it was discovered that individual biomarker publications (encompassing genetic, protein or various blood biomarkers) often reported larger effect estimates for hypothesized associations between the biomarker and the disease outcome. In contrast, the effect estimate proved to be much smaller than the initial estimate when the same biomarker was later compared in the meta-analyses [83]. In addition, there is the issue that many different extraction techniques exist for the extraction and detection of peripheral miRNAs – supporting the need for the development of optimal common procedures across laboratories. Scalability and input material requirements are among the major disadvantages of sequencing-based approaches.

PCR amplification is one of the steps in sample preparation. There is a loss of information during PCR analysis, should it be over-performed, which can occur consequent to excessive duplication. The ability to scale down starting material requirements is particularly vital in cases where the amount of input material is limited (such as in biomarker development for human blood or CSF samples) [79]. Due to the fact that the technology is quickly evolving, there is currently a lack of consensus amongst scientists with regard to the optimal approach to analyze large-scale miRNA profiles. Nevertheless, notwithstanding ongoing limitations in research, the detection and quantification of specific miRNA biomarkers may serve as a minimally invasive and relatively inexpensive screening test to allow early detection of NDDs, such as AD and PD [84]. With further research and a greater knowledge of the miRNAs involved in the progression of AD and PD, we may gain the opportunity to develop better and earlier clinical care for patients and to design and evaluate more effective therapeutic interventions by ultimately targeting specific miRNAs or miRNA signature profiles that underpin the progression of AD and PD.

Acknowledgments

This work was supported in part by the Intramural Research Program of the National Institute on Aging, NIH.

List of abbreviations

AD	Alzheimer's disease
APP	Amyloid precursor protein
β-amyloid	beta-amyloid
BBB	blood-brain barrier
CSF	cerebrospinal fluid
DA	dopaminergic neurons
LRRK2	Leucine-rich repeat kinase 2
miRNAs	microRNAs
NDDs	neurodegenerative disorders
PD	Parkinson's disease
PBMCs	peripheral blood mononuclear cells
SPT	Serine palmitoyltransferase

References

1. Maffioletti E, Tardito D, Gennarelli M, Bocchio-Chiavetto L. Micro spies from the brain to the periphery: new clues from studies on microRNAs in neuropsychiatric disorders. *Front Cell Neurosci.* 2014; 8:75. [PubMed: 24653674]
2. Johnson R, Noble W, Tartaglia GG, Buckley NJ. Neurodegeneration as an RNA disorder. *Prog Neurobiol.* 2012; 99:293–315. [PubMed: 23063563]

3. Goodall EF, Heath PR, Bandmann O, Kirby J, Shaw PJ. Neuronal dark matter: the emerging role of microRNAs in neurodegeneration. *Front Cell Neurosci.* 2013; 7:178. [PubMed: 24133413]
4. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. *Nat Rev Genet.* 2007; 8(2):93–103. [PubMed: 17230196]
5. Cuellar TL, McManus MT. MicroRNAs and endocrine biology. *J Endocrinol.* 2005; 187(3):327–332. [PubMed: 16423811]
6. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature.* 2005; 433(7027):769–773. [PubMed: 15685193]
7. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science.* 2004; 303(5654):83–6. [PubMed: 14657504]
8. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, et al. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature.* 2004; 432(7014):226–30. [PubMed: 15538371]
9. Kamal MA, Mushtaq G, Greig NH. Current update on synopsis of miRNA deregulation in Neurological Disorders. *CNS Neurol Disord Drug Targets.* 2015; 13:8.
10. Tan L, Yu JT, Tan L. Causes and Consequences of MicroRNA Dysregulation in Neurodegenerative Diseases. *Mol Neurobiol.* 2014 Epub ahead of print.
11. Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS. A microRNA array reveals extensive regulation of microRNAs during brain development. *RNA.* 2003; 9:1274–1281. [PubMed: 13130141]
12. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science.* 2001; 294:853–858. [PubMed: 11679670]
13. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholak H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. Serum microRNAs are promising novel biomarkers. *PLoS One.* 2008; 3:e3148. [PubMed: 18773077]
14. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem.* 2010; 285:17442–17452. [PubMed: 20353945]
15. Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, Qin YW, Jing Q. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J.* 2010; 31:659–666. [PubMed: 20159880]
16. Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res.* 2010; 38:7248–7259. [PubMed: 20615901]
17. De Smaele E, Ferretti E, Gulino A. MicroRNAs as biomarkers for CNS, cancer and other disorders. *Brain Res.* 2010; 1338:100–11. [PubMed: 20380821]
18. Alzheimer's Association. 2010 Alzheimer's Disease: Facts and Figures. Alzheimer's Association; Chicago, IL: 2010.
19. Mushtaq G, Khan JA, Kamal MA. Biological Mechanisms linking Alzheimer's disease and Type 2 Diabetes Mellitus. *CNS Neurol Disord Drug Targets.* 2014; 13(4):1192–1201. [PubMed: 25230227]
20. Klein WL. Synaptic targeting by A beta oligomers (ADDLS) as a basis for memory loss in early Alzheimer's disease. *Alzheimer's Dement.* 2006; 2(1):43–55. [PubMed: 19595855]
21. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science.* 2002; 298(5594):789–791. [PubMed: 12399581]
22. Walsh DM, Selkoe DJ. Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron.* 2004; 44(1):181–193. [PubMed: 15450169]
23. Thomas T, Thomas G, McLendon C, Sutton T, Mullan M. beta-Amyloid-mediated vasoactivity and vascular endothelial damage. *Nature.* 1996; 380(6570):168–171. [PubMed: 8600393]
24. Aliev G, Priyadarshini M, Reddy VP, Grieg NH, Kaminsky Y, Cacabelos R, et al. Oxidative Stress Mediated Mitochondrial and Vascular Lesions as Markers in the Pathogenesis of Alzheimer Disease. *Curr Med Chem.* 2014; 21(19):2208–2217. [PubMed: 24372221]

25. Walsh DM, Selkoe DJ. A beta oligomers - a decade of discovery. *J Neurochem.* 2007; 101:1172–1184. [PubMed: 17286590]
26. Stoicea N, Wilson T, Chandrasekhar K, Bergese SD. The Role of microRNAs as Potential Biomarkers in Central Nervous System Disorders. *Int J Clin Anesthesiol.* 2013; 1:1009.
27. Danborg PB, Simonsen AH, Waldemar G, Heegaard NH. The potential of microRNAs as biofluid markers of neurodegenerative diseases--a systematic review. *Biomarkers.* 2014; 19(4):259–68. [PubMed: 24678935]
28. Hebert SS, De Strooper B. Alterations of the microRNA network cause neurodegenerative disease. *Trends Neurosci.* 2009; 32:199–206. [PubMed: 19268374]
29. Schonrock N, Gotz J. Decoding the non-coding RNAs in Alzheimer's disease. *Cell Mol Life Sci.* 2012; 69:3543–3559. [PubMed: 22955374]
30. Hebert SS, Horre K, Nicolai L, Papadopoulou AS, Mandemakers W, Silahtaroglu AN, et al. Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. *Proc Natl Acad Sci USA.* 2008; 105:6415–6420. [PubMed: 18434550]
31. Hebert SS, Horre K, Nicolai L, Bergmans B, Papadopoulou AS, Delacourte A, et al. MicroRNA regulation of Alzheimer's Amyloid precursor protein expression. *Neurobiol Dis.* 2009; 33:422–428. [PubMed: 19110058]
32. Schonrock N, Ke YD, Humphreys D, Staufenbiel M, Ittner LM, Preiss T, et al. Neuronal MicroRNA Deregulation in Response to Alzheimer's Disease Amyloid- β . *PLoS ONE.* 2010; 5(6):e11070. [PubMed: 20552018]
33. Lukiw WJ. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. *Neuroreport.* 2007; 18(3):297–300. [PubMed: 17314675]
34. Cogswell JP, Ward J, Taylor IA, Waters M, Shi Y, Cannon B, et al. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. *J Alzheimers Dis.* 2008; 14:27–41. [PubMed: 18525125]
35. Burgos K, Malenica I, Metpally R, Courtright A, Rakela B, et al. Profiles of Extracellular miRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology. *PLoS ONE.* 2014; 9(5):e94839. [PubMed: 24797360]
36. Alexandrov PN, Dua P, Hill JM, Bhattacharjee S, Zhao Y, Lukiw WJ. MicroRNA (miRNA) speciation in Alzheimer's disease (AD) cerebrospinal fluid (CSF) and extracellular fluid (ECF). *Int J Biochem Mol Biol.* 2012; 3:365–373. [PubMed: 23301201]
37. Schipper HM, Maes OC, Chertkow HM, Wang E. MicroRNA expression in Alzheimer blood mononuclear cells. *Gene Regul Syst Bio.* 2007; 1:263–274.
38. Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, Huang Q, et al. The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. *J Neurosci.* 2008; 28:1213–1223. [PubMed: 18234899]
39. Lukiw WJ, Zhao Y, Cui JG. An NF-kappa-B sensitive microRNA-146a mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. *J Biol Chem.* 2008; 283:31315–31322. [PubMed: 18801740]
40. Sethi P, Lukiw WJ. Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. *Neurosci Lett.* 2009; 459:100–104. [PubMed: 19406203]
41. Müller M, Kuiperij HB, Claassen JA, Kusters B, Verbeek MM. MicroRNAs in Alzheimer's disease: differential expression in hippocampus and cell-free cerebrospinal fluid. *Neurobiol Aging.* 2014; 35:152–158. [PubMed: 23962497]
42. Leidinger P, Backes C, Deutscher S, Schmitt K, Muller SC, Frese K, et al. A blood based 12-miRNA signature of Alzheimer disease patients. *Genome Biol.* 2013; 14(7):R78. [PubMed: 23895045]
43. Sheinerman KS, Tsivinsky VG, Crawford F, Mullan MJ, Abdullah L, Umansky SR. Plasma MicroRNA biomarkers for detection of mild cognitive impairment. *Aging (Albany, NY).* 2012; 4:590–605. [PubMed: 23001356]

44. Geekiyanage H, Chan C. MicroRNA-137/181c Regulates Serine Palmitoyltransferase and in turn Amyloid {beta}, Novel Targets in Sporadic Alzheimer's Disease. *J Neurosci*. 2001; 31:14820–14830.
45. Geekiyanage H, Jicha GA, Nelson PT, Chan C. Blood serum miRNA: Non-invasive biomarkers for Alzheimer's disease. *Exp Neurol*. 2012; 235(2):491–496. [PubMed: 22155483]
46. Kumar P, Dezso Z, MacKenzie C, Oestreicher J, AgoulNIK S, et al. Circulating miRNA Biomarkers for Alzheimer's Disease. *PLoS ONE*. 2013; 8(7):e69807. [PubMed: 23922807]
47. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders? *Neurology*. 2007; 68:326–337. [PubMed: 17261678]
48. Thomas B, Beal MF. Parkinson's disease. *Hum Mol Genet*. 2007; 16(S2):R183–R194. [PubMed: 17911161]
49. Goetz CG. The history of Parkinson's disease: Early clinical descriptions and neurological therapies. *Cold Spring Harb Perspect Med*. 2011; 1(1):a008862. [PubMed: 22229124]
50. Shitlans A, Henchcliffe C. Biomarkers in Parkinson's disease: an update. *Curr Opin Neurol*. 2012; 25:460–465. [PubMed: 22772875]
51. Schapira AH. Pathogenesis of Parkinson's disease. *Baillieres Clin Neurol*. 1997; 6:15–36. [PubMed: 9426866]
52. Mouradian MM. MicroRNAs in Parkinson's disease. *Neurobiol Dis*. 2012; 46:279–284. [PubMed: 22245218]
53. Ferrer I, Martinez A, Blanco R, Dalfo E, Carmona M. Neuropathology of sporadic Parkinson disease before the appearance of parkinsonism: preclinical Parkinson disease. *J Neural Transm*. 2011; 118:821–839. [PubMed: 20862500]
54. Maciotta S, Meregalli M, Torrente Y. The involvement of microRNAs in neurodegenerative diseases. *Front Cell Neurosci*. 2013; 7:265. [PubMed: 24391543]
55. Grünblatt E. Parkinson's disease: molecular risk factors. *Parkinsonism Relat Disord*. 2012; 18(Suppl 1):S45–8. [PubMed: 22166452]
56. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997; 388(6645):839–840. [PubMed: 9278044]
57. Sotiropoulos S, Gibney G, Baxevanis AD, Nussbaum RL. A single nucleotide polymorphism in the 3'UTR of the SNCA gene encoding alpha-synuclein is a new potential susceptibility locus for Parkinson disease. *Neurosci Lett*. 2009; 461:196–201. [PubMed: 19540308]
58. Doxakis E. Post-transcriptional regulation of alpha-synuclein expression by miR-7 and miR-153. *J Biol Chem*. 2010; 285:12726–12734. [PubMed: 20106983]
59. Junn E, Lee KW, Jeong BS, Chan TW, Im JY, Mouradian MM. Repression of alpha-synuclein expression and toxicity by microRNA-7. *Proc Natl Acad Sci USA*. 2009; 106:13052–13057. [PubMed: 19628698]
60. Alvarez-Erviti L, Seow Y, Schapira AH, Rodriguez-Oroz MC, Obeso JA, Cooper JM. Influence of microRNA deregulation on chaperone-mediated autophagy and alpha-synuclein pathology in Parkinson's disease. *Cell Death Dis*. 2013; 4:e545. [PubMed: 23492776]
61. Winslow AR, Rubinshtein DC. The Parkinson disease protein alpha-synuclein inhibits autophagy. *Autophagy*. 2011; 7:429–431. [PubMed: 21157184]
62. Margis R, Margis R, Rieder CR. Identification of blood microRNAs associated to Parkinson's disease. *J Biotechnol*. 2011; 152:96–101. [PubMed: 21295623]
63. Cardo LF, Coto E, DeMena L, Ribacoba R, Moris G, Menendez M, et al. Profile of microRNAs in the plasma of Parkinson's disease patients and healthy controls. *J Neurol*. 2013; 260:1420–1422. [PubMed: 23543376]
64. Martins M, Rosa A, Guedes L, Fonseca B, Gotovac K, Violante S, et al. Convergence of miRNA expression profiling, α -synuclein interactome and GWAS in Parkinson's disease. *PLoS ONE*. 2011; 6(10):e25443.
65. Soreq L, Salomonis N, Bronstein M, Greenberg D, Israel Z, Bergman H, et al. Small RNA sequencing-microarray analyses in Parkinson leukocytes reveal deep brain stimulation-induced splicing changes that classify brain region transcriptomes. *Front Mol Neurosci*. 2013; 6:10. [PubMed: 23717260]

66. Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, et al. A MicroRNA feedback circuit in midbrain dopamine neurons. *Science*. 2007; 317:1220–1224. [PubMed: 17761882]
67. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet*. 2009; 41:1303–1307. [PubMed: 19915576]
68. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet*. 2009; 41:1308–1312. [PubMed: 19915575]
69. Lin X, Parisiadou L, Gu XL, Wang L, Shim H, Sun L, Xie C, Long CX, Yang WJ, Ding J, et al. Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein. *Neuron*. 2009; 64:807–827. [PubMed: 20064389]
70. Cho HJ, Liu G, Jin SM, Parisiadou L, Xie C, Yu J, et al. MicroRNA-205 regulates the expression of Parkinson's disease-related leucine-rich repeat kinase 2 protein. *Hum Mol Genet*. 2013; 22(3): 608–20. [PubMed: 23125283]
71. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011; 377:1019–1031. [PubMed: 21371747]
72. Becker RE, Seeman MV, Greig NH, Lahiri DK. What can triumphs and tribulations from drug research in Alzheimer's disease tell us about the development of psychotropic drugs in general? *Lancet Psychiatry*. 2015; 2(8):756–64. [PubMed: 26249306]
73. Becker RE, Greig NH, Giacobini E, Schneider LS, Ferrucci L. A new roadmap for drug development for Alzheimer's disease. *Nat Rev Drug Discov*. 2014; 13(2):156. [PubMed: 24362362]
74. Dougall NJ, Bruggink S, Ebmeier KP. Systematic review of the diagnostic accuracy of 99mTc-HMPAO-SPECT in dementia. *Am J Geriatr Psychiatry*. 2004; 12:554–570. [PubMed: 15545324]
75. Nordberg A. Dementia in 2014. Towards early diagnosis in Alzheimer disease. *Nat Rev Neurol*. 2015; 11(2):69–70. [PubMed: 25623789]
76. Patwardhan MB, McCrory DC, Matchar DB, Samsa GP, Rutschmann OT. Alzheimer disease: operating characteristics of PET—a meta-analysis. *Radiology*. 2004; 231:73–80. [PubMed: 15068942]
77. Gallivanone F, Rosa PA, Castiglioni I. Statistical voxel-based methods and [18F]FDG PET brain imaging: frontiers for the diagnosis of AD. *Curr Alzheimer Res*. 2015 Epub ahead of print.
78. Eldh M, Lotvall J, Malmhall C, Ekstrom K. Importance of RNA isolation methods for analysis of exosomal RNA: evaluation of different methods. *Mol Immunol*. 2012; 50:278–286. [PubMed: 22424315]
79. Rao P, Benito E, Fischer A. MicroRNAs as biomarkers for CNS disease. *Front Mol Neurosci*. 2013; 6:39. [PubMed: 24324397]
80. Burgos K, Javaheria A, Bompreszi R, Ghaffari L, Rhodes S, Courtright A, et al. Identification of extracellular miRNA in human cerebrospinal fluid by next-generation sequencing. *RNA*. 2013; 19:712–722. [PubMed: 23525801]
81. Rong H, Liu T, Yang K, Yang H, Wu D, Liao C, et al. MicroRNA-134 plasma levels before and after treatment for bipolarmania. *J Psychiatr Res*. 2011; 45:92–95. [PubMed: 20546789]
82. Ioannidis J, Allison D, Ball C, Coulibaly I, Cui X, Culhane A, et al. Repeatability of published microarray gene expression analyses. *Nat Genet*. 2009; 41:149–155. [PubMed: 19174838]
83. Ioannidis J, Panagiotou O. Comparison of effect sizes associated with biomarkers reported in highly cited individual articles and in subsequent meta-analyses. *JAMA*. 2011; 305:2200–2210. [PubMed: 21632484]
84. Grasso M, Piscopo P, Confaloni A, Denti MA. Circulating miRNAs as biomarkers for neurodegenerative disorders. *Molecules*. 2014; 19(5):6891–910. [PubMed: 24858274]

Table 1

Summary of studies on microRNAs in biofluids as biomarkers in AD

Name/Signature of miRNAs	Patient Population	Type of biological material	Observations / Results	References
61 different miRNAs dysregulated	69 AD patients and 78 neurologically healthy controls	serum and CSF	41 miRNAs were significantly dysregulated in the CSF of AD patients compared to controls while 20 miRNAs were expressed differently in the serum of AD patients compared to controls	35
miR-34a and miR-181b	16 AD patients and 16 normal elderly controls	PBMCs	miR-34a and miR-181b were found to be significantly upregulated	37
miR-137, miR-9, miR-29a/b and miR-181c	7 probable AD patients compared to 7 healthy controls	human serum	circulating levels of were found to be significantly lower in the blood serum of probable AD patients compared to healthy controls	45
let-7g-5p, let-7d-5p, miR-142-3p, miR-15b-5p, miR-545-3p, miR-191-5p, miR-301a-3p	11 AD patients and 20 healthy human subjects	human plasma	This distinct circulating 7-miRNA signature was found to be significantly down-regulated in AD patients	46
60 different miRNAs dysregulated including miR-210, miR-9, miR-125b, all members of the miR-30 family	6 AD patients and 9 healthy human controls	CSF	Sixty different miRNAs were found to be dysregulated between early stage AD and late-stage AD	29
miR-146a	20 AD patients and 22 healthy human subjects	CSF	miR-146a was found to be markedly decreased in the CSF of AD patients.	41
miR-9, miR-34a, miR-125b, mmiR-155, miR-28 and miR-146a	6 AD patients and 6 healthy subjects	CSF	miR-9, miR-34a, miR-125b, mmiR-155, miR-28 and miR-146a levels were significantly higher in AD patients compared to controls	36
12-miRNA signature	94 AD patients and 21 healthy subjects	Whole Blood	12-miRNA signature was detected in AD patients (has-let-7d-3p, let-7f-5p, miR-26a-5p, miR-26b-5p, miR-103a-3p, miR-107, miR-5020-3p, miR-112, miR-161, miR-151a-3p, miR-1285-5p, miR-532-5p)	42
Two sets of miRNA pairs	20 AD patients, 20 MCI patients and 20 cognitively normal subjects	human plasma	Two sets of miRNA pairs (miR-323-3p/-370, miR-134/-370 and miR-382/-370) and (miR-132/-491-5p, miR-128/-491-5p and miR-874/-491-5p) distinguished AD and MCI patients from control subjects but not from each other	43

List of abbreviations:

Alzheimer disease (AD), cerebrospinal fluid (CSF), microRNA (miRNA), Mild Cognitive Impairment (MCI), peripheral blood mononuclear cells (PBMCs)

Table 2

Summary of studies on microRNAs in biofluids as biomarkers in PD

Name/Signature of miRNAs	Patient Population	Type of biological material	Observations / Results	References
miR-1, miR-22*, miR-29a	8 untreated PD patients, eight healthy, control subjects	blood samples	These three miRNAs were detected in the blood of PD patients compared to control subjects	62
miR-137, miR-193a-3p, miR-125a-3p, miR-196b, miR-454miR-181c, miR-331-5p	25 healthy, control persons and 31 untreated PD patients	human plasma	These seven miRNAs were found to be upregulated in PD patients	63
17 different miRNAs including let-7, miR-128, miR-433, miR-485-5p, miR-212, miR-132, miR-338-3p, miR-30e-3p, miR-30a-3p, miR-16-2-3p	57 PD patients and 65 healthy controls	serum and CSF	17 miRNAs were significantly dysregulated in the CSF of PD patients while 5 miRNAs were expressed differently in the serum of PD patients compared to controls	35
18 different miRNAs including miR-30b, miR-30c and miR-26a	19 PD patients and 13 healthy control subjects	PBMCs	18 miRNA were found to be dysregulated	64
16 different miRNAs including miR-20a, miR-16-1/2, miR-15b, miR-320-b-1/2, miR-378, miR-320a, miR-769	7 PD patients (prior to and after deep brain stimulation) and 6 healthy, control subjects	leukocytes	16 miRNAs differentially expressed in PD patients compared to control subjects; 11 miRNAs expression pattern changed after deep brain stimulation indicating a reversal of miRNA pattern to healthy after stimulation	65

List of abbreviations:

cerebrospinal fluid (CSF), microRNA (miRNA), messengerRNA (mRNA), Parkinson's disease (PD), peripheral blood mononuclear cells (PBMCs)