Published in final edited form as:

Neurobiol Dis. 2012 January ; 45(1): 76–82. doi:10.1016/j.nbd.2010.10.022.

Current status and future directions of gene expression profiling in Parkinson's disease

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Abstract

Parkinson's disease (PD) is a common age-associated neurodegenerative disorder. Motor symptoms are the cardinal component of PD, but non-motor symptoms, such as dementia, depression, and autonomic dysfunction are being increasingly recognized. Motor symptoms are primarily caused by selective degeneration of substantia nigra dopamine (SNDA) neurons in the midbrain; non-motor symptoms may be referable to well-described pathology at multiple levels of the neuraxis. Development of symptomatic and disease-modifying therapies is dependent on an accurate and comprehensive understanding of the pathogenesis and pathophysiology of PD. Gene expression profiling has been recently employed to assess function on a broad level in the hopes of gaining greater knowledge concerning how individual mechanisms of disease fit together as a whole and to generate novel hypotheses concerning PD pathogenesis, diagnosis, and progression. So far, the majority of studies have been performed on postmortem brain samples from PD patients, but more recently, studies have targeted enriched populations of dopamine neurons and have begun to explore extra-nigral neurons and even peripheral tissues. This review will provide a brief synopsis of gene expression profiling in parkinsonism and its pitfalls to date and propose several potential future directions and uses for the technique. It will focus on the use of microarray experiments to stimulate hypotheses concerning mechanisms of neurodegeneration in PD, since the majority of studies thus far have addressed that complicated issue.

Keywords

Parkinson's; gene expression; microarray; pathogenesis; mitochondria; axon; axon guidance; synaptic function; synuclein

Parkinson's disease (PD) is a common neurodegenerative disorder afflicting about 1% of people over age 55. The cardinal signs of the disease are motor in nature and include resting tremor, bradykinesia, rigidity, and postural instability. These motor symptoms primarily result from selective degeneration of substantia nigra dopamine (SNDA) neurons in the midbrain.

Recently, there has been a rediscovery of the fact that PD is not merely a specific disorder of SNDA neurons, but a more systemic neurological disease that affects multiple levels of the

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neuraxis, including the cortex, amygdala, brainstem, peripheral autonomic nervous system (sympathetics and parasympathetics), and the enteric nervous system (ENS) lining the gastrointestinal (GI) tract. Symptoms are similarly varied and encompass multiple non-motor features, including cognitive impairment, dementia, depression, sleep disturbance, autonomic instability, and digestive dysfunction (Adler, 2005; Barone et al., 2009; Martinez-Martin et al., 2007; Poewe, 2007; Temlett and Thompson, 2006).

Development of symptomatic treatments for motor and non-motor symptoms, as well as any potential disease-modifying (neuroprotective) therapies, is dependent on an accurate and comprehensive understanding of the pathogenesis and pathophysiology of PD. The majority of studies addressing such issues have been hypothesis-driven 'candidate-mechanism' approaches. Scientifically, this approach is the only way to test and delineate specific mechanisms of disease and therapeutic intervention; however, it is an approach that is necessarily limited by previous experience and current scientific understanding. In some cases, it may engender asking questions that are the most answerable, not necessarily the most relevant. The search for unique and unexpected factors impacting the pathophysiology of PD and many other diseases has led to the development of systems approaches that attempt to assess function on a broader level in the hopes of gaining greater knowledge concerning how individual components fit together as a whole.

One such method is gene expression profiling, which has been touted as a way of generating new hypotheses concerning the pathogenesis of Parkinson's disease, enhancing diagnostic accuracy, improving predictions about progression and prognosis, and predicting disease in asymptomatic individuals (Miller and Federoff, 2006; Papapetropoulos et al., 2007). For example, one recent experiment used SNDA neuron expression profiling to search for novel genetic loci for vulnerability to PD and reported an association, although two subsequent studies failed to replicate the finding (Elstner et al., 2009; Guella et al., 2010; Vilarino-Guell et al., 2010). So far, the majority of studies have been performed on the midbrain and striatum in postmortem samples from PD patients and animal models of parkinsonism (Bassilana et al., 2005; Bossers et al., 2009; Duke et al., 2007; Grunblatt et al., 2004; Hauser et al., 2005; Miller et al., 2004; Miller et al., 2006; Moran et al., 2006; Papapetropoulos et al., 2006; Sutherland et al., 2009; Zhang et al., 2005). More recently, studies have targeted enriched populations of dopamine neurons (as opposed to tissue pieces) and have begun to explore extra-nigral neurons and peripheral tissues (Cantuti-Castelvetri et al., 2007; Meurers et al., 2009; Mutez et al., 2010; Scherzer et al., 2007; Simunovic et al., 2009; Simunovic et al., 2010; Stamper et al., 2008; Yacoubian et al., 2008). This review will provide an overview of gene expression profiling in parkinsonism and its pitfalls to date and propose several potential future directions and uses for the technique. It will focus on the use of microarray experiments to stimulate hypotheses concerning the causes of PD, since the majority of studies thus far have addressed that complicated issue.

Global assessment of gene expression in postmortem samples from PD patients

Several microarray experiments have been performed using postmortem brain samples from PD patients. A feature all of the studies share in common is the ability to segregate samples into appropriate groups based on gene expression profile alone (Bossers et al., 2009; Cantuti-Castelvetri et al., 2007; Duke et al., 2007; Grunblatt et al., 2004; Hauser et al., 2005; Miller et al., 2006; Moran et al., 2006; Papapetropoulos et al., 2006; Simunovic et al., 2009; Sutherland et al., 2009; Zhang et al., 2005). Samples from PD patients are consistently differentiated from those obtained from matched control individuals without evidence of neurological disease. In addition, one study has suggested that expression profiles are disease-selective in that segregation is fairly consistent between PD, progressive

supranuclear palsy (PSP), and frontotemporal dementia with parkinsonism (FTD-P) (Hauser et al., 2005). Distinction has also been made based on the presence or absence of dementia in PD (Stamper et al., 2008). Different brain regions (SN v. striatum; lateral v. medial SN) from PD patients can also be correctly categorized based on expression profile (Duke et al., 2007; Miller et al., 2006; Moran et al., 2006). Furthermore, there are strong gender-related differences in alterations of gene expression associated with PD (Cantuti-Castelvetri et al., 2007; Simunovic et al., 2010).

Clearly, PD affects gene expression in a global way in multiple cell types (discussed below). This is a very interesting finding, but its significance is not clear at present. Is it a pathological signature, like SNDA neuron loss or Lewy bodies? Does it provide clues to mechanisms of neurodegeneration? Is it a result of circuitry or neuronal activity changes? Is it a more complicated interaction, such as the way a PD patient responds to end-of-life issues, such as respiratory failure? There are a myriad of interpretive questions arising from this data, and they are not easily answered experimentally. A few will be highlighted below with consideration of potential next steps. Regardless, the data appear to indicate that PD affects global gene expression in a stereotypic way. Understanding the reasons for and implications of this broad-based change in transcriptional neuroanatomy would be extremely useful in understanding PD as a whole.

Analysis of individual transcripts and molecular pathways in gene expression data from PD

The ultimate utility of describing global alterations in gene expression in PD will be decided based on its therapeutic impact. For instance, will analysis of transcription profiles indicate an unexpected and effective target for a pharmacological or genetic intervention to retard the progression of PD or improve its treatment? With this in mind, analysis of expression data in PD has focused on examination of individual genes and related groups of genes with the idea being that an individual gene or concerted pathway might provide an opening for disease-modifying therapeutics.

Differences in individual genes have not been consistent between experiments. In fact, a recent meta-analysis of several PD microarray studies revealed that no individual gene showed reliably different expression among the 11 studies evaluated, even after a re-analysis of 6 of the datasets using a common method (Sutherland et al., 2009). The absence of a handful of dramatically altered transcripts has been somewhat disappointing from the perspective of rapid discovery and intervention, but is not surprising given the heterogeneous and complex nature of PD phenotypes.

It seems more intuitive to suspect it is not a single or handful of transcripts, but multiple differentially expressed genes that account for functional differences in PD. Additionally, it has been shown experimentally in several studies that examination of related gene sets (or pathways) produces more consistent results between experiments than does comparison of lists of individually different transcripts (Subramanian et al., 2005; Ye and Eskin, 2007). As such, this analysis technique seems a more powerful and sensitive method for overcoming biological variability (between individuals and series of individuals), experimental noise, and inter-laboratory differences that complicate and cloud the interpretation of microarray experiments.

In fact, analysis of concerted molecular pathways in gene expression data from PD patients has been more revealing, but correlation between individual studies remains poor (Sutherland et al., 2009). Those studies performed have tended to highlight previously hypothesized mechanisms of PD pathogenesis. For example, Grunblatt et al in 2004 described decreases in protein modification and degradation pathways, energy metabolism,

and signal transduction and increases in cytoskeleton, cell cycle, and stress pathways (Grunblatt et al., 2004). In a similar experiment, protein handling and degradation (ubiquitin pathways, chaperones), mitochondrial function, vesicle trafficking, and apoptotic pathways were implicated in substantia nigra from PD patients, but not from PSP or FTD. However, there were only three non-PD patients in that study, and no statistical analysis was performed to test the strength of the pathway differences (Hauser et al., 2005). The general idea has been relatively similar in several studies of PD substantia nigra, with the implication of protein handling/mishandling, synaptic function, neurotrophins, axonal functions, and energy pathways being recurring themes (Table 1) (Bossers et al., 2009; Duke et al., 2007; Miller et al., 2006; Moran et al., 2006; Papapetropoulos et al., 2006; Zhang et al., 2005).

While several confounding factors (such as disease stage, sample composition, and cell-specific gene expression) are discussed in more detail below, a few more comments are warranted as to why data from different PD microarray experiments are weakly correlated with one another even when pathways are considered instead of individual transcripts. Varied patient populations within and between studies (clinical characteristics, medications, degree/stage of pathology, environmental exposure history etc.) are most likely a significant issue; this includes biological variability in the samples and also variability in clinical or pathological assessment and sample preparation. Different array platforms and analytical techniques were particularly problematic in early microarray experiments, although more contemporary studies have alleviated many of those concerns (Yauk and Berndt, 2007). Small numbers of cases have plagued PD microarray experiments and contributed to poor inter-experiment correlation.

Recent studies have begun to compare results using more sophisticated approaches, and combining samples from multiple populations and platforms may be a viable future technique for detecting more reliable signals (Sutherland et al., 2009). An international collaborative group (the Global PD Gene Expression (GPEX) Consortium) has taken such an approach and recently completed a tour-de-force meta-analysis of 17 independent expression studies in PD that will be discussed in more detail below (Zheng et al., 2010).

Impact of sample composition on gene expression data in PD: cell type and basal gene expression

One critical question is what is actually being evaluated in microdissected pieces of SN (or other regions) from PD patients (Figure 1). Even under control circumstances, SN samples are extremely heterogeneous with multiple cell types (DA neurons, non-DA neurons, astrocytes, microglia, oligodendrocytes, peripheral blood cells, etc.) represented in different proportions depending on the dissection technique. In PD, there are dramatic pathological changes that make the situation even more confusing. Loss of SNDA neurons occurs to varying degrees. Astrocytes and microglia are not only activated, but also recruited to the site. Some breakdown of the blood-brain barrier may also occur. These changes make interpretation of gene expression results very problematic. In particular, while changes are often interpreted to mean a particular gene or pathway is involved in the pathogenesis of PD or SNDA neuron death, it is much more likely that the changes observed are a result of the pathology and not the cause.

For example, one fairly consistent alteration noted in earlier studies of SN pieces was a substantial decrease in transcripts related to dopamine signaling pathways (Table 1; (Miller and Federoff, 2005; Miller and Federoff, 2006; Sutherland et al., 2009)). This might be interpreted to mean that these pathways may be a causal or contributing factor to the pathophysiology of PD. In reality, these alterations are almost certainly an artifact of significant dopamine neuron loss in the PD substantia nigra.

Several studies have attempted to minimize the impact of neuron loss by examining more homogeneous populations of SNDA neurons collected using laser-capture microdissection (LCM) (Cantuti-Castelvetri et al., 2007; Simunovic et al., 2009). This technique allows for analysis of a highly-enriched sample of SNDA neurons from patients and control individuals and has proven successful to a certain degree in PD and animal models of PD. The assumption is that using enriched neuronal populations instead of regional microdissections greatly increases the sensitivity with which changes can be detected, and thus the likelihood of generating interpretable results. Recently, we have directly demonstrated this to be correct in the case of rodent models of PD, where dramatic alterations in expression of metabolic genes during complex I inhibition was only observed in SNDA neuron samples, not in the midbrain as a whole (Fig 1) (Greene et al., 2010).

Microarray studies of SNDA neurons obtained by LCM have provided some interesting insights to complement examination of tissue pieces from PD patients. In particular, suspected alterations in dopamine signaling pathways have not been found in homogeneous populations of SNDA neurons, confirming suspicion they are secondary to neuronal loss. Other pathways originally described as active in tissue pieces, such as synaptic transmission, signal transduction, and mitochondrial function remain prominent in LCM samples (Cantuti-Castelvetri et al., 2007; Simunovic et al., 2009).

Although it is intuitive on the surface to expect similar categorical changes in nigral tissue (whole) and SNDA neurons (homogeneous) in PD, it raises some concern about misleading analyses. Pathway based analyses typically compare sets of differentially regulated genes from a certain specific anatomical area (SN, SNDA neurons, etc.) with the comparator set being all genes in the genome. When that is the case, it is difficult to know if the 'overrepresentation' is due to the pathology, or merely the difference in tissues being examined. For example, are synaptic and mitochondrial gene sets different in LCM SNDA neurons from PD patients due to the disease process, or merely due to the fact that neurons in general express those genes more robustly than the average cell in the body?

As another example, expression profiling has indicated that inflammation-related genes and those involved in leukocyte extravasation are altered in leukocytes from LRRK2 carriers with parkinsonism (Mutez et al., 2010). The result was interpreted to implicate leukocyte functions in PD pathogenesis, which is certainly possible. However, another consideration is that alteration of leukocyte genes in leukocytes by any genetic mutation is predictable. By definition, leukocyte-related transcripts are the only ones expressed by leukocytes, and that pathway may or may not have anything to do with PD.

A more appropriate comparator for pathways analysis, not often used, is the genes expressed normally in that particular cell type. In conjunction with more general analysis, comparing within the same tissue or cell type could provide more specific information about differences related to PD itself, not confounded by cell-type differences.

Interestingly, LCM studies have demonstrated a dramatic impact of gender on SNDA neuron gene expression pattern at baseline and in the setting of PD (Cantuti-Castelvetri et al., 2007; Simunovic et al., 2010). Most notably, the transcript differences that have been described between PD patients and controls in SNDA neurons appear to come from two separate gene sets with minimal overlap, an interesting finding given that men have 1.5-2 fold higher risk for developing PD (Van Den Eeden et al., 2003; Wooten et al., 2004). Essentially, the genes that are altered in SNDA neurons in PD are different in male and female patients. Pathway analysis has indicated a similar finding, whereby functional categories overrepresented in male PD cases are different than those overrepresented in female cases (Simunovic et al., 2010).

This brings up another important point concerning the impact of basal gene expression level on changes caused by PD or PD-related insults. In particular, individual or even systematic expression changes in PD are potentially seriously misleading to the point of irrelevance if considered in the absence of baseline expression differences between cell types.

For example, we have recently described a dramatic bimodal change in energy metabolism transcripts in DA neurons in rotenone-treated rats and MPTP-treated mice (Greene et al., 2010). The magnitude of the changes was substantially greater in SNDA neurons as compared to VTADA neurons by more than 2-fold (Figure 1). This is consistent with the well-described overabundance of energy metabolism transcripts in SNDA neurons at baseline. We have interpreted this to mean that in their metabolically active state, inhibiting mitochondrial output produces a more profound relative metabolic deficit in SNDA neurons and causes a more dynamic transcriptional response. However, another possible interpretation is that transcripts that are expressed in a given cell type are the most likely to be affected by any insult. In other words, if a cell type expresses genes ABC, those are much more likely to be affected than genes XYZ that are not as prominent.

Considering basal differences, especially gender and cell type, will be important in future profiling experiments in PD. Luckily, the burgeoning field of transcriptional neuroanatomy continues to provide new and important information about gene expression profiles under normal conditions (Lein et al., 2007; Lu et al., 2006; Luo et al., 1999).

Potential impact of disease stage on interpretation of gene expression results

Every expression profiling experiment performed to date on neural tissue from PD patients has employed postmortem tissue samples. The large majority are from PD patients in advanced stages of disease. This raises significant issues concerning interpretation, especially with regard to disease pathogenesis.

As discussed above, pathological changes in the composition of the midbrain in advanced PD patients make it difficult to know what expression differences signify. RNA changes seen in advanced cases may not be reflective in the least of what occurs in early PD due to the differing and variable abundance of cell types in the PD nigra.

LCM of homogenous populations of SNDA neurons can correct to a certain degree for concerns about sample composition, but interpretive hurdles remain. For instance, surviving SNDA neurons captured from advanced PD patients may be a unique population of cells that are less vulnerable. They may also have engaged effective compensatory mechanisms resulting in survival. As such, the expression profile in these cells may actually be one indicating survival, not impending damage or susceptibility to damage (Cantuti-Castelvetri et al., 2007). Conversely, the cells may exhibit significant signs of damage, such as α -synuclein pathology (Lu et al., 2005). In that case, the 'end-stage' expression profile may be one of impending degeneration that is still not relevant as far as disease-modifying intervention is concerned.

Theoretically, the ideal neuron population to tease out causes of neurodegeneration in PD would be one much earlier in the disease course. Of course, practical issues make this is easier said than done. Capturing sufficient numbers of the types of postmortem samples required (e.g., SN from very early PD or asymptomatic gene carriers) will require a long-term international collaboration, but the potential insights and benefit to the field of PD research are enormous.

Expression profiling of non-nigral tissues

Moving beyond late-stage PD patients is a critical step for gene expression profiling to meet its full potential as a hypothesis generator for PD. Expression profiling from extra-nigral tissues is attractive for several reasons. First, many symptoms of PD are not referable to the SN or the rest of the basal ganglia. These include non-motor symptoms such as dementia, sleep disturbance, autonomic instability, and depression, as well as some motor complaints like freezing and falling. Second, PD pathology defined as either α -synuclein neuritic pathology or neuronal loss is prominent in many areas outside the nigra, including the brainstem and periphery (Beach et al., 2010; Braak et al., 2003). These findings raise the specter that focusing on the substantia nigra to the exclusion of other central and peripheral neural tissues results in an incomplete and potentially misleading picture of PD pathology and pathogenesis. Third, neural (and non-neural) tissues in the periphery are more accessible for biopsy in living patients. Access is a critical issue because it would allow longitudinal analysis of individual patients from the earliest presymptomatic stages to advanced stages of PD. Accessibility and suitability of the ENS in living PD patients has recently been demonstrated (Lebouvier et al., 2008; Lebouvier et al., 2010).

Expression profiling of cortex, striatum, and cerebellum has already yielded some interesting results, although they remain subject to several of the caveats discussed above. Interestingly, the majority of studies have report a much greater degree of transcriptional changes in the striatum than the nigra. It might be interpreted as dysregulation, but a more conservative theory is that it is a response to denervation (Miller et al., 2005; Miller et al., 2006; Vogt et al., 2006). It may not be as important for elucidating causes of SNDA death, but it could still be a critical point in pathogenesis and a highly attractive therapeutic target. Normalizing key transcripts may help to normalize striatal function and improve PD symptoms.

Profiling of cortical cells has provided insight into dementia in PD (PDD) and also corroborated some results from SN in PD and animal models. Gene expression profiling in laser captured cingulate cortical neurons accurately separated PDD patients from non-demented PD patients and controls (Stamper et al., 2008). Several pathways were identified as potential contributors to the cause of dementia in PD, since they were progressively altered in patients who were cognitively normal and those who were demented. These pathways included axonal function and synaptic transmission, both have which have been implicated from dopaminergic samples.

Two studies have examined expression profiles of blood in PD patients; one used whole blood and the other isolated peripheral blood mononuclear cells (Mutez et al., 2010; Scherzer et al., 2007; Soreq et al., 2008). A very interesting finding from those experiments, and one that may profoundly impact our consideration of PD as a disease, is that there are significant changes in leukocytes associated with PD. Much like the data from brain samples, expression profiles from white blood cells provide sufficient information to segregate PD cases and controls. Several biochemical pathways, such as protein chaperones, programmed cell death, axon guidance pathways, and inflammation have been highlighted in the two studies, corroborating data from brain samples and suggesting these pathway disruptions may be systemic in nature. Of particular interest is the finding in the Mutez study that an asymptomatic LRRK2 carrier segregated with parkinsonian patients with the LRRK2 mutation (Mutez et al., 2010). Since the majority of patients enrolled in both studies were in earlier stages of PD, the data are intriguing from the standpoint of pathogenesis (Figure 2).

The next frontier for gene expression analysis in PD would seem to be evaluation of peripheral neural tissues in live PD patients. The ENS, particularly the submucosal plexus, is

amenable to biopsy. This is most typically performed in the rectum in children as the diagnostic test for Hirschsprung's disease (aganglionosis of the GI tract). Submucosal biopsy has been reported twice to detect phosphorylated α -synuclein deposition in PD patients (Lebouvier et al., 2008; Lebouvier et al., 2010). Technical considerations, including neuron yield, would have to be overcome, but this type of experiment may provide some very interesting information.

Even in the absence of evaluation in early living patients, examination of expression profiles of neurons in the ENS, sympathetic ganglia, dorsal motor nucleus of the vagus and other regions in the same patient could provide very instructive information concerning the ways different neuron phenotypes respond to PD, especially when compared to SNDA neurons.

A collaborative approach to gene expression profiling in PD

During revision of this review, a novel and important collaborative profiling study was reported implicating a master transcriptional regulator of bioenergetics, PGC-1 α (peroxisome proliferator-activated receptor γ coactivator-1 α), in the pathogenesis of PD (Zheng et al., 2010). The study analyzed variation in expression of multiple members of molecular pathways using GSEA (Gene Set Enrichment Analysis) by a random-effects model meta-analysis of 17 studies (five previously unpublished, others discussed above). The raw data (image files) were analyzed (or re-analyzed) and processed using a standard algorithm. The analysis encompassed 221 PD samples and 189 controls, and it was performed in three stages. The first stage included six studies of whole substantia nigra and three of DA neurons captured by LCM. This design minimized differences related solely to loss of DA neurons (sample composition) and identified 28 gene sets (out of 522) altered in PD. The second stage validated 12 of the 28 gene sets in a cohort of patients with early subclinical disease; these patients did not have a diagnosis of PD in life but were found to have incidental Lewy bodies on neuropathological examination, thus minimizing the impact of disease stage on the results. The third stage took the analysis to extra-nigral tissues (including blood) and validated 10 of the 12 surviving sets. These 10 gene sets showed a significant association with PD in the same direction (downregulated) at every stage of analysis.

All ten gene sets were related to bioenergetics and focused on only four inter-related categories: electron transport, mitochondrial biogenesis, glucose utilization, and glucose-sensing. Quantitative real-time PCR was used to validate the down-regulation of 19 individual electron transport transcripts (10 responsive to PGC-1 α) in a new population of substantia nigra samples from 13 PD patients and 17 controls. Q-RT-PCR also confirmed that all 19 genes examined were down-regulated in incidental Lewy body cases (possible preclinical PD).

This approach addresses many of the confounding issues raised during analysis and presentation of the individual expression studies. Issues of sample size, sample composition, basal expression, disease stage, and extra-nigral PD involvement have been tackled fairly effectively. The whole of this analysis is clearly more than the sum of its parts, and it has provided a critical clue to the pathogenesis of PD.

Insights into PD pathogenesis from mammalian models of parkinsonism

One of the benefits to working with animal models is the chance to overcome issues associated with advanced pathology by examining asymptomatic or early stage animals. Other advantages include a more homogenous population of subjects treated in nearly identical ways, a clearly defined insult (genetic abnormality or toxic exposure), defined behavioral and pathological outcomes, and sample availability. As with any animal

experiment, the faithfulness with which the model recapitulates the human disease is critical. One contribution of expression analysis to the study of PD could be to provide a barometer to measure how accurately individual animal models replicate PD and are appropriate for study.

Animal models currently have the advantage for examining earlier points on the temporal continuum of parkinsonism. One study has used LCM and microarrays to examine early toxicity in rotenone-treated rats by evaluating animals with mild motor impairment in the absence of SNDA loss (Meurers et al., 2009). Interestingly, this experiment described changes in transcripts related to cell death suggesting that early on SNDA neurons mount a transcriptional response to compensate for rotenone-induced mitochondrial toxicity. RNAs related to transcriptional control were also significantly affected.

We have gone further with this design and evaluated rotenone-treated rats that were behaviorally normal, albeit using RT-PCR of selected transcripts, not global profiling. Interestingly, we found a biphasic response in SNDA neurons in rotenone-treated animals, whereby asymptomatic rats had overall increases in energy metabolism transcripts, while functionally-impaired animals had decreases (Greene et al., 2010). These results suggest that employing more broadly-based transcriptional assessments very early in the course of parkinsonism may be profitable in animal models.

Results using the neurotoxin MPTP have been somewhat different in that earlier time points have tended to display more robust alterations in gene expression. In particular, one day after systemic MPTP, transcripts related to cytoskeletal maintenance and synaptic function are decreased in whole SN from mice (Miller et al., 2004). Other experiments, including our own, have described early changes after MPTP in mice which are probably a result of early neurodegeneration and damage in these models (Greene et al., 2010; Mandel et al., 2002).

Evaluation of MPTP-treated monkeys has yielded some interesting results (Bassilana et al., 2005; Ohnuki et al., 2010). Depending on analysis method, there is some concordance between MPTP intoxication and later stage PD, especially in the areas of synaptic function, cytoskeleton, and transcriptional regulation. Evaluating the time course of MPTP intoxication has proven instructive, as different transcripts are regulated at different points of chronic MPTP intoxication corresponding to different degrees of DA neuron loss (Bassilana et al., 2005). At earlier points, developmental and dopaminergic transcripts are actually increased, suggesting a compensatory response. One MPTP study has suggested the involvement of two specific transcription factors, REST and the androgen receptor in later stage dopaminergic degeneration (Ohnuki et al., 2010). In general, regarding pathogenic interpretations, MPTP experiments suffer from the same limitations as studies of advanced human PD. The overlap between MPTP and human PD is encouraging, but whether it signifies a common response to neuron death or a real indicator of pathological mechanism remains to be determined.

Alpha-synuclein transgenic mice have also contributed to the study of global gene expression in parkinsonism. Mice overexpressing wild-type human α -synuclein in most neurons (PDGF promoter) have few nigral expression changes at 3 months of age when there is minimal pathology; however, expression changes are more robust in older animals. The categories most notably affected were transcripts relevant to transcription and DNA binding (Yacoubian et al., 2008). Analysis of mice expressing α -synuclein behind the tyrosine hydroxylase (TH) promoter showed some nigral expression changes at young age, including upregulated dopamine signaling and synaptic genes, correlating with changes seen in early MPTP toxicity in primates (Miller et al., 2007). The assumption is that study was

that TH-synuclein mice represented early parkinsonism, but that was not confirmed in older mice.

Given the absence of appropriate material from PD patients, several groups have compared information from different animal models with that from patients in an attempt to determine the time course of expression changes in parkinsonism. Despite the inherent flaws in this approach, several overarching themes appear to recur (Bassilana et al., 2005; Miller and Federoff, 2005; Miller and Federoff, 2006; Ohnuki et al., 2010). Most have suggested an initial increase in transcripts related to dopamine signaling and synaptic function; energy metabolism might be added to that group based on more recent data. Inflammatory transcripts as a group tend to be more affected in advanced disease. Other contributors, such as protein metabolism and degradation, cell-to-cell signaling, cell death, and neurotrophins have been altered in both early and late models. More work is critical in this area to help fill in the skeletal timeline we suggest in Figure 2.

In general, animal models have provided interesting results to complement expression profiling from PD patients, potentially filling in some gaps relevant to early disease and causes of neurodegeneration. However, interpretation is based on the tenuous assumption that the animal model systems examined replicate degenerative mechanisms in PD.

Conclusions and future directions

The promise of gene expression as an unbiased method of determining potential causes of PD is unfulfilled at present, but the outlook is improving. Previously proposed candidate mechanisms have been highlighted, which is encouraging, but completely novel pathways have not. This may be because those novel pathways do not exist. It may also be because the sampling strategies and analysis techniques employed thus far are merely scratching the surface of options available. The latter is at least suggested by the lack of concordance between individual expression profiling experiments focused on PD. Innovative analytical and collaborative methods may maximize information gleaned from expression analyses already completed (Zheng et al., 2010).

Critical next steps should include extensive analyses of defined cell populations, including glial cells, as well as correlative analyses of multiple different central and peripheral neural tissues. In addition to novel samples, further analysis of different patient populations, including very early stage or 'presymptomatic' PD patients will be important. In particular, patients at genetic risk for developing PD, LRRK2 carriers for instance, may provide a fruitful line of investigation (Mutez et al., 2010). Conversely, use of gene expression profiles as an endophenotype may identify new genetic associations for PD (AD paper and failed replication paper). A caveat to that direction of investigation is that it will add another layer of complexity to the analyses and interpretation, since it is by no means clear that mechanisms of pathogenesis are shared between genetic and sporadic PD. Longitudinal analysis using enteric biopsies or leukocytes is likely to provide a wealth of information simply not obtainable in cross-sectional post-mortem studies and should be a high priority.

Combination of transcriptome analysis with genome, proteome and 'metabolome' analyses has nearly limitless potential, although it will be extraordinarily complex to perform such a study appropriately. Performing the experiments in a collaborative and comprehensive fashion will increase the likelihood of generating interpretable results and identifying new therapeutic targets in PD.

Acknowledgments

This work was supported by NINDS K08 NS048858 and a George C. Cotzias Memorial Fellowship from the American Parkinson Disease Association.

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Figure 1. Impact of sample composition and timing on gene expression profile

A. Photomicrograph of mouse midbrain stained for tyrosine hydroxylase (TH). Samples evaluated in parkinsonism (humans and mammalian models) have included whole midbrain, whole substantia nigra, SNC (substantia nigra pars compacta), including differentiation between medial (med) and lateral (lat). Dashed circles represent a sample of SNDA neurons obtainable by LCM. VTA, ventral tegmental area; SNR, substantia nigra pars reticulata. **B.** The graph depicts gene expression changes in the rotenone model of parkinsonism in rats with the ordinate axis being the composite expression change (vs. vehicle-treated) of five energy metabolism transcripts (ND1, NADH dehydrogenase subunit ND1; COX1, cytochrome c oxidase subunit 1; LDHB, lactate dehydrogenase B; TPI, triose phosphate isomerase 1; PDHC, pyruvate dehydrogenase subunit 1). There are four groups of animals: vehicle, very early (1 week of rotenone treatment), early (four weeks of treatment with no symptoms), and mid (symptomatic, with or without mild neurodegeneration). Note that sample composition impacts results, with dramatic differences between microdissected midbrain and laser-captured SNDA and VTADA neurons. Note also the substantial effect of time (disease stage) on the results from DA neurons.

Figure 2. Possible alterations in gene expression in parkinsonism

Theoretical schematic of PD progression depicts progressive damage from striatal dopamine terminals to SNDA cell bodies, including Lewy body formation, cell loss, and activation of microglia and astrocytes. Nigral gene expression patterns are below based on mammalian models and PD cases. Remember the caveat that correlation between gene expression experiments in PD is poor so far.

Table 1

Molecular pathways deregulated in postmortem PD samples

Cellular pathway	Studies highlighting pathway
Mitochondria/energy/electron transport	6 out of 9
Protein degradation	6 out of 9
Synaptic transmission/axons/vesicle trafficking	6 out of 9
Cell signaling/signal transduction	5 out of 9
Dopamine signaling	3 out of 9
Apoptosis/cell death	3 out of 9
Neurotrophin	2 out of 9

Overview of pathways highlighted and discussed by nine different studies that examined gene expression profiles from postmortem samples from PD. All were microarray experiments that used RNA from either whole substantia nigra (Bossers et al., 2009; Duke et al., 2007; Grunblatt et al., 2004; Hauser et al., 2005; Miller et al., 2006; Papapetropoulos et al., 2006; Zhang et al., 2005) or SNDA neurons obtained by LCM (Cantuti-Castelvetri et al., 2007; Simunovic et al., 2009) as input.