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Finding Diamonds in the Rubble

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Abstract

A recent publication by Vogt and colleagues illustrates both the power and pitfalls of using gene expression profiling of neurodegenerative disease (24).

‘Neurodegeneration’ is a generic term which describes the death of neurons, and, as such, could be applied to acute injury such as brain trauma. In contrast, the term ‘neurodegenerative disease’ connotes those disorders in which an endogenous process produces dysfunction and death of neurons over the course of years. In the past decade, there has been increasing recognition of the remarkable similarities among the neurodegenerative diseases (2,13,17, 20). For many of these disorders, genetic forms of the disease have been identified (6,8,12, 19,23). For many of these disorders, there are characteristic proteinaceous aggregates: amyloid plaques in Alzheimer's disease, Lewy bodies in Parkinson's disease (PD), neuronal intranuclear inclusions in Huntington's disease, Bunina bodies in amyotrophic lateral sclerosis, and glial cytoplasmic inclusions in multiple system atrophy (MSA) and progressive supranuclear palsy. Interestingly, it is not known if these characteristic proteins aggregates are in themselves toxic, or if they represent a compensatory cellular response. Another recent realization is that abnormal symptoms may not result purely from neuronal death, but may reflect abnormal functioning of the remaining neurons. Thus, defining the scope of neuronal dysfunction is critical in unraveling these disorders.

Brain cells – neurons – are unique in that these post-mitotic cells encode experience-dependent alterations in their activity, so-called synaptic plasticity. Whereas a decade ago, the prevailing theory was that synaptic plasticity was a phenomenon that occurred primarily at the synapse, there is increasing realization that transcriptional events are critical to the encoding and maintenance of synaptic plasticity. What neurons do is alter their activity in response to external signals; we now understand that this type of lifelong plasticity depends on a continual interrogation of the genome. Transcription is the process whereby cells read specified portions of the DNA. Thus, increasing evidence demonstrates that neurons depend on transcriptional events in order to function normally.

Expression profiling is the technique that permits simultaneous assessment of expression levels of thousands of genes. Using DNA microarrays, one can obtain an instantaneous snapshot of cellular activity. These measurements can often lead to novel hypotheses regarding pathogenesis. The first application of DNA microarrays to human neurologic disease revealed gene expression abnormalities in a transgenic mouse model of Huntington's disease (HD) (15). Subsequently, gene expression abnormalities have been found in a number of neurodegenerative diseases, including AD (1), ALS (5), PD (9,10,16), prion diseases (25), frontotemporal dementia and PSP (10), and multiple sclerosis (22), suggesting that impacting the genome is another common feature of these disorders. These studies of human

neurodegenerative disease reveal some general principles. As a general rule, in neurodegenerative disease, there are circumscribed sets of gene expression changes, typically involving about only 1-5% of expressed transcripts. That is, the expression level of most genes is not changed. In addition, there is regional specificity of gene changes in neurodegenerative diseases. For example, in human HD, gene expression changes are most prominent in the caudate and motor cortex, with far fewer changes detectable in the cerebellum and prefrontal cortex (11). Interestingly, the gene changes found among different neurodegenerative conditions are distinct; these gene expression alterations do not simply reflect chronic brain disease. The implication is that, by sifting through the tea leaves of microarray data, one might be able to discern disease-specific mechanisms.

In the current issue, Vogt *et al.* examine the transcriptional changes in the putamen in two neurodegenerative diseases, multiple systems atrophy (MSA) and Parkinson's disease (PD). These syndromes share clinical and pathological features, with MSA traditionally having classified as one of the 'Parkinson's plus' syndromes. Both are characterized pathologically by cytoplasmic inclusions comprised in part by α -synuclein: Lewy bodies in PD and glial cytoplasmic inclusions in MSA. Familial forms of PD have been identified, and corresponding transgenic mouse models have been produced. However, transgenic mice expressing mutant forms of α -synuclein fail to reproduce key features of PD pathology; most notably, these mice do not manifest dopaminergic cell death (reviewed in 7). Transgenic mouse models for MSA have been developed, although there are significant limitations in each (reviewed in 21). Where they exist, transgenic mice offer significant advantages for mRNA expression profiling, with less variability of medication exposure and post-mortem interval. Thus, transcriptional profiling of transgenic mice offers better signal-to-noise over using post-mortem human brain material. However, when a suitable animal model is not available, the best option is the use of well-characterized post-mortem human brain.

The current study measures transcriptional changes in human brain. The authors specifically excluded known familial cases of PD. Degradation of macromolecules, especially of RNA, is a well-known result of prolonged postmortem interval. Vogt *et al.* have controlled carefully for tissue pH, a measure that correlates well with the integrity of mRNA; RNA degrades quickly at acid pH levels. The authors have used an imaginative approach in the current study. Since there is damage to the nigrostriatal pathway in both PD and MSA, they postulated that transcripts in the putamen regulated in a similar manner would reflect nigrostriatal damage, whereas transcripts that are differentially regulated in the putamen might be disease-specific. Since the cerebellum is unaffected in PD, the authors reasoned that altered transcripts in the cerebellum might be specific for MSA pathology. The authors also examined gene transcription profiles in occipital cortex, with the assumption that this area is unaffected in either disorder.

One unavoidable limitation of the approach is the reliance on human neuropathology. The definition of which areas are considered 'affected' and 'unaffected' is based largely on the basis of neuronal loss and/or gliosis. That is, subtle pathology, such as gene dysregulation, that does not produce neuronal death, would be missed by this pathological criterion. In all pathology-guided studies, we are necessarily biased towards those areas that manifest the highest amounts of neuronal death. One might imagine that the occipital cortex is not in fact unaffected, but simply does not manifest as many obvious changes as other areas. The authors have subtracted the occipital cortex gene changes from the putamen and cerebellum, aware of the possibility that this manipulation may miss some important changes. However, in an initial study such as this, it is quite defensible to focus on the areas that one believes are undeniably affected. It is worth noting, that for both PD and MSA cases, there were actually more genes differentially expressed (compared to control cases) in the occipital cortex, followed by the cerebellum, with the putamen actually demonstrating the fewest number of differentially expressed genes. These observations call into the question whether occipital cortex can be

considered truly 'unaffected' in MSA and PD. A reassessment of the neuropathological gold standard of neuronal death may be called for. One might imagine that some brain regions may actually show gene expression changes than those manifesting the greatest degree of cell death. For example, in Huntington's disease transgenic mice, there is markedly reduced expression of NR2a and NR2b subunits of the NMDA receptor in the hippocampus, a region considered to be affected to a lesser degree than the striatum (14). Downregulation of these subunits may render the hippocampus relatively protected from glutamate excitotoxicity. So, if cell death is the downstream event, it may not faithfully parallel the magnitude of upstream pathology.

In terms of the current study, there is good news and bad news. The good news is there are a lot of data. The bad news is that...there are a lot of data. As with any gene expression profiling study, there are as many interesting hypotheses generated as questions answered.

Prior PD gene expression studies have focused on the substantia nigra pars compacta, the area in which the cell bodies of the susceptible dopaminergic neurons reside. Grunblatt et al. demonstrated distinctive alterations in ubiquitin-proteasome, heat shock proteins, iron- and oxidative stress, cell adhesion, and vesicle trafficking (9). In the current study, gene alterations in the putamen are quite different, confirming that in PD, there is not a generalized gene dysregulation. Location matters: gene expression changes in the dopaminergic source areas differ from the dopaminergic target areas. This important regional difference echoes why it is imperative to solve these problems in intact brain. Such anatomically-specific effects would be impossible to model in a cell culture system. Next, in these non-Mendelian PD cases, there was no difference in the expression of α -synuclein, suggesting that alteration of α -synuclein expression levels is not a factor in sporadic PD.

Despite some clinical and pathologic similarities, there is little overlap in the gene expression profiles between MSA and PD in either the putamen or cerebellum. So, on a molecular level, these disorders are distinct, a conclusion that is hardly surprising to clinical neurologists. What might be surprising is just how distinct the expression profiles are. Clinical phenotypes may be relatively restricted. For example, while mutations in several genes cause clinical Alzheimer disease, the clinical picture is remarkably homogeneous. Only four genes were regulated in both MSA and PD putamen, while only 8 genes were upregulated both in MSA and PD cerebellum. In the putamen, only two transcripts were regulated in the same direction in both MSA and PD: neuropeptide Y (downregulated) and heterogeneous ribonucleoprotein C1/2 (upregulated). Serotonin receptor 2C (HTR2C) and ataxin-1 (SCA-1) were both upregulated in MSA putamen and downregulated in PD putamen. The observation of altered expression of a serotonin receptor is interesting, given the link between serotonin signaling and tremor (18). Neuropeptide Y is one of the transmitter peptides that modulate signaling within the caudate-putamen. Recent advances in the development of neuropeptide agonists and antagonists raise the possibility that augmenting neuropeptide Y function might be beneficial in these disorders (3). This type of scenario illustrates the power of using gene expression studies in generating novel hypotheses. These studies, however, must be interpreted with caution, as a prior study had shown increased neuropeptide Y expression in PD striatum (4).

Which brings us to the problem. Despite the considerable power of the DNA microarray approach, these studies are essentially descriptive. While 'descriptive' is often used by journal reviewers as a pejorative term, I point out only that gene expression studies can tell one which genes are changed but not *why* those genes are changed. Nevertheless, these *descriptive* studies produce huge amounts of data, unthinkable in the pre-array era.

Where to go from here? In the post-array era, increasingly scientists are forced to wrestle with the cause and effect question. Beyond the critical issues of statistical rigor, most of which have been routinized by now, the larger issue is how to interpret the changes that one can measure.

As a colleague asked me, “Can you imagine any biological process where gene expression doesn't change?” In other words, do these gene changes cause the disease, or are they simply the downstream effect of the pathogenic insult? This type of question may not be amenable to being answered by using post-mortem human tissue. One can reformulate new hypotheses into testable questions, to be addressed in *in vitro* models of transgenic mice. If one hypothesizes that a gene change is relevant in the pathogenesis of a neurodegenerative disease, then experimentally preventing that change should ameliorate the disease phenotype. Similarly, artificially introducing that change in gene expression—for example, through transient transfection in a cell culture model—should reproduce some of the disease phenotype. These studies can show whether the observed gene change is *necessary* and *sufficient* to produce the disease phenotype. All of these studies are a lot of work, but may yield insights into a class of currently untreatable diseases. If scientific inquiry is akin to mining, powerful high-throughput techniques such as gene expression profiling is the dynamite that smashes the side of a mountain into small manageable pieces. It is up to us now to do the hard work of sifting through the rubble in order to find the diamonds.

References

1. Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW. Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci U S A* 2004;101:2173–2178. [PubMed: 14769913]
2. Bossy-Wetzel E, Schwarzenbacher R, Lipton SA. Molecular pathways to neurodegeneration. *Nat Med* 2004;10(Suppl):S2–9. [PubMed: 15272266]
3. Brain SD, Cox HM. Neuropeptides and their receptors: innovative science providing novel therapeutic targets. *Br J Pharmacol* 2006;147(Suppl 1):S202–211. [PubMed: 16402106]
4. Cannizzaro C, Tel BC, Rose S, Zeng BY, Jenner P. Increased neuropeptide Y mRNA expression in striatum in Parkinson's disease. *Brain Res Mol Brain Res* 2003;110:169–176. [PubMed: 12591154]
5. Dangond F, Hwang D, Camelo S, Pasinelli P, Frosch MP, Stephanopoulos G, Brown RH Jr, Gullans SR. Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter. *Physiol Genomics* 2004;16:229–239. [PubMed: 14645737]
6. Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. *Nat Rev Genet* 2006;7:306–318. [PubMed: 16543934]
7. Fernagut PO, Chesselet MF. Alphasynuclein and transgenic mouse models. *Neurobiol Dis* 2004;17:123–130. [PubMed: 15474350]
8. Goedert M, Jakes R. Mutations causing neurodegenerative tauopathies. *Biochim Biophys Acta* 2005;1739:240–250. [PubMed: 15615642]
9. Grunblatt E, Mandel S, Jacob-Hirsch J, Zeligson S, Amariglio N, Rechavi G, Li J, Ravid R, Roggendorf W, Riederer P, Youdim MB. Gene expression profiling of parkinsonian substantia nigra pars compacta; alterations in ubiquitinproteasome, heat shock protein, iron and oxidative stress regulated proteins, cell adhesion/cellular matrix and vesicle trafficking genes. *J Neural Transm* 2004;111:1543–1573. [PubMed: 15455214]
10. Hauser MA, Li YJ, Xu H, Noureddine MA, Shao YS, Gullans SR, Scherzer CR, Jensen RV, McLaurin AC, Gibson JR, Scott BL, Jewett RM, Stenger JE, Schmechel DE, Hulette CM, Vance JM. Expression profiling of substantia nigra in Parkinson disease, progressive supranuclear palsy, and frontotemporal dementia with parkinsonism. *Arch Neurol* 2005;62:917–921. [PubMed: 15956162]
11. Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RL, Olson JM, Jones L, Luthi-Carter R. Regional and cellular gene expression changes in human Huntington's disease brain. *Hum Mol Genet* 2006;15:965–977. [PubMed: 16467349]
12. Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is unstable in Huntington's disease chromosomes. *Cell* 1993;72:971–983. [PubMed: 8458085]
13. Kosik KS, Shimura H. Phosphorylated tau and the neurodegenerative foldopathies. *Biochim Biophys Acta* 2005;1739:298–310. [PubMed: 15615647]

14. Luthi-Carter R, Apostol BL, Dunah AW, DeJohn MM, Farrell LA, Bates GP, Young AB, Standaert DG, Thompson LM, Cha J-HJ. Complex alteration of NMDA receptors in transgenic Huntington's disease mouse brain: analysis of mRNA expression levels, protein abundance, interacting proteins and phosphorylation. *Neurobiology of Disease* 2003;14:624–636. [PubMed: 14678777]
15. Luthi-Carter R, Strand A, Peters NL, Solano SM, Hollingsworth ZR, Menon AS, Frey AS, Spektor BS, Penney EB, Schilling G, Ross CA, Borchelt DR, Tapscott SJ, Young AB, Cha J-HJ, Olson JM. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum Mol Genet* 2000;9:1259–1271. [PubMed: 10814708]
16. Miller RM, Callahan LM, Casaceli C, Chen L, Kiser GL, Chui B, Kaysser-Kranich TM, Sendera TJ, Palaniappan C, Federoff HJ. Dysregulation of gene expression in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse substantia nigra. *J Neurosci* 2004;24:7445–7454. [PubMed: 15329391]
17. Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* 2005;6:11–22. [PubMed: 15611723]
18. Nicholson SL, Brotchie JM. 5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease - opportunities for novel therapeutics to reduce the problems of levodopa therapy. *Eur J Neurol* 2002;9 (Suppl 3):1–6. [PubMed: 12464115]
19. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, Brown RH, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis [published erratum appears in *Nature* 1993 Jul 22;364(6435):362] [see comments]. *Nature* 1993;362:59–62. [PubMed: 8446170]
20. Roy S, Zhang B, Lee VM, Trojanowski JQ. Axonal transport defects: a common theme in neurodegenerative diseases. *Acta Neuropathol (Berl)* 2005;109:5–13. [PubMed: 15645263]
21. Stefanova N, Tison F, Reindl M, Poewe W, Wenning GK. Animal models of multiple system atrophy. *Trends Neurosci* 2005;28:501–506. [PubMed: 16043239]
22. Steinman L, Zamvil S. Transcriptional analysis of targets in multiple sclerosis. *Nat Rev Immunol* 2003;3:483–492. [PubMed: 12776208]
23. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 2005;120:545–555. [PubMed: 15734686]
24. Vogt IR, Lees AJ, Evert BO, Klockgether T, Bonin M, Wullner U. Transcriptional changes in multiple system atrophy and Parkinson's disease putamen. *Exp Neurol* 2006;199:465–478. [PubMed: 16626704]
25. Xiang W, Windl O, Wunsch G, Dugas M, Kohlmann A, Dierkes N, Westner IM, Kretschmar HA. Identification of differentially expressed genes in scrapie-infected mouse brains by using global gene expression technology. *J Virol* 2004;78:11051–11060. [PubMed: 15452225]