Expression Profiling of Substantia Nigra in Parkinson Disease, Progressive Supranuclear Palsy, and Frontotemporal Dementia With Parkinsonism

Michael A. Hauser, PhD; Yi-Ju Li, PhD; Hong Xu, MA; Maher A. Noureddine, PhD; Yujun S. Shao, PhD; Steven R. Gullans, PhD; Clemens R. Scherzer, MD; Roderick V. Jensen, PhD; Adam C. McLaurin, BA; Jason R. Gibson, BA; Burton L. Scott, MD; Rita M. Jewett, RN; Judith E. Stenger, PhD; Donald E. Schmechel, MD; Christine M. Hulette, MD, PhD; Jeffery M. Vance, MD, PhD

Background: Parkinson disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra. Genes contributing to rare mendelian forms of PD have been identified, but the genes involved in the more common idiopathic PD are not well understood.

Objectives: To identify genes important to PD pathogenesis using microarrays and to investigate their potential to aid in diagnosing parkinsonism.

Design: Microarray expression analysis of postmortem substantia nigra tissue.

Patients: Substantia nigra samples from 14 unrelated individuals were analyzed, including 6 with PD, 2 with progressive supranuclear palsy, 1 with frontotemporal dementia with parkinsonism, and 5 control subjects.

Main Outcome Measures: Identification of genes significantly differentially expressed (P<.05) using Affymetrix U133A microarrays.

Results: There were 142 genes that were significantly differentially expressed between PD cases and controls and 96 genes that were significantly differentially expressed between the combined progressive supranuclear palsy and frontotemporal dementia with parkinsonism cases and controls. The 12 genes common to all 3 disorders may be related to secondary effects. Hierarchical cluster analysis after exclusion of these 12 genes differentiated 4 of the 6 PD cases from progressive supranuclear palsy and frontotemporal dementia with parkinsonism.

Conclusions: Four main molecular pathways are altered in PD substantia nigra: chaperones, ubiquitination, vesicle trafficking, and nuclear-encoded mitochondrial genes. These results correlate well with expression analyses performed in several PD animal models. Expression analyses have promising potential to aid in postmortem diagnostic evaluation of parkinsonism.

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cal records were reviewed by a movement specialist (B.L.S.) to ensure that the subjects met previously reported criteria. Lewy body pathologic evidence was evaluated according to consensus guidelines and PSP according to National Institute of Neurological Disorders and Stroke criteria. Frontotemporal dementia with parkinsonism linked to chromosome 17q21-22 has been described. All 9 patient samples showed typical pathologic features, including moderate to severe neuronal loss and gliosis. Control subjects were cognitively normal, died of non-neurological causes, and had no clinical or pathological evidence of a movement disorder.

PROCUREMENT OF RNA

At autopsy, brain hemispheres were frozen in liquid nitrogen and stored at −80°C in the Kathleen Price Bryan Brain Bank in the Alzheimer’s Disease Research Center at Duke University. Using the RNAgents kit (Promega, Madison, Wis), RNA was extracted from SN and adjacent midbrain tissues. The delay before postmortem examinations varied (Table 1); however, brain messenger RNA is stable for up to 36 hours after death. Double-stranded complementary DNA was made with a biotinylated T7(dT)-24 primer.

MICROARRAYS

Twenty micrograms of biotinylated complementary RNA was fragmented and hybridized to Affymetrix human genome U133A microarrays (Affymetrix Inc, Santa Clara, Calif). Affymetrix Microarray Suite 5.0 software was used for global scaling, with a mean “target intensity” of 100 for all probe sets. To control for partial RNA degradation, 3/5/’ ratios for glyceraldehyde-3-phosphate dehydrogenase probes were examined (M33197_5_at and M33197_3_at). Of 19 original samples, 5 (1 PD, 3 control, and 1 PSP) with 3/5/’ end ratios greater than 5.0 were excluded from analysis.

DATA ANALYSIS

We analyzed 1164 probe sets with mean intensities of at least 500. After log transforming the raw intensities, differentially expressed genes were identified using a 2-sample t test. This study was hypothesis generating rather than hypothesis testing, so we report nominal P values with α = .05. Supervised hierarchical clustering was performed using Cluster (http://rana.lbl.gov/EisenSoftware.htm) with the complete linkage option and visualized using TreeView (http://rana.lbl.gov/EisenSoftware.htm). Affymetrix hybridization probes were mapped to genomic linkage peaks as previously described.

Table 1. Tissue Donor Information

<table>
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<tr>
<th>Sample No.</th>
<th>Diagnosis</th>
<th>Age, y</th>
<th>Sex</th>
<th>PD Braak Stage</th>
<th>AD Braak Stage</th>
<th>Postmortem Delay, h:min</th>
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<td>673</td>
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<td>F</td>
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<td>NA</td>
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<tr>
<td>543</td>
<td>Control</td>
<td>72</td>
<td>F</td>
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<td>M</td>
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<td>87</td>
<td>M</td>
<td>III</td>
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<td>PD</td>
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<td>M</td>
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<tr>
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<td>F</td>
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<tr>
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<td>M</td>
<td>III</td>
<td>IV</td>
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<td>M</td>
<td>V</td>
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<tr>
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<tr>
<td>718</td>
<td>FTDP</td>
<td>58</td>
<td>F</td>
<td>NA</td>
<td>NA</td>
<td>24:00</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; FTDP, frontotemporal dementia with parkinsonism; NA, not available; PD, Parkinson disease; PSP, progressive supranuclear palsy.

RESULTS

Affymetrix U133A chips were used to measure SN gene expression from 6 PD, 2 PSP, 1 FTDP, and 5 control samples. First, the 6 PD samples were compared with the 5 control samples, revealing 142 (122 reduced and 20 elevated) significantly differentially expressed genes (P < .05) (a table containing this supplemental information is available from the corresponding author). Fold changes (≥4-fold) are consistent with those seen in other investigations. Table 2 gives a subset of these genes that fall into molecular pathways previously associated with PD. This differential expression has been confirmed using serial analysis of gene expression. The 142 genes and others in the same pathways are candidates for PD susceptibility and phenotypic modifier genes, and will be tested by association analysis in patients with PD and controls.

The SN of patients with PD shows many secondary effects of disease (eg, neuronal loss and gliosis) that may induce expression changes unrelated to disease cause or progression. The PSP and FTDP samples analyzed also show loss of dopaminergic neurons and should exhibit the same secondary effects. We identified 96 genes that were significantly differentially expressed between PSP and FTDP cases and controls (P < .05) (a table containing this supplemental information is available from the corresponding author). Twelve of these genes were also differentially expressed between PD and control SN (Figure, A). We hypothesize that these genes reflect secondary effects common to all 3 disorders and should be given less priority in the search for genes involved in PD pathogenesis, leaving 130 prioritized genes. Twenty of these 130 genes map to regions of PD linkage (a table containing this supplemental information is available from the corresponding author).
the corresponding author). These are potential PD susceptibility genes, as they are functional (expression) and positional (linkage) candidates.

Finally, we explored the potential to use gene expression to place the samples into diagnostic groups. We used 226 genes (142 PD vs control, and 84 PSP/H11001 FTDP vs control) (Figure, A) to perform supervised hierarchical clustering. Although this was unsuccessful (Figure, B), after removing the 12 secondary effect genes, the samples fell into 3 distinct clusters, with only a single PD sample that was misclassified (Figure, C).

Our global expression profiling of neural tissue from PD, PSP, and FTDP patients identified 130 prioritized candidate genes, correlating well with expression studies of model systems for PD (Table 3). It also identified 12 genes that may reflect secondary changes due to neuronal loss. Despite the limited sample size, removal of these genes increased the specificity of supervised hierarchical clustering, suggesting that a formal clas-
We demonstrate increases in heat shock proteins HSPA1A and HSPA1B in PD, PSP, and FTDP compared with control SN, indicating that this may be a common response to mitigate the toxic effects of misfolded protein. This is supported by the ability of Hsp70 to reverse the phenotype of the α-synuclein transgenic fly and by the up-regulation of endogenous chaperones in R406W microtubule-associated protein tau (MAPT) flies.

Mutations in the ubiquitination genes UCHL1 (PARK5) and parkin (PARK2) have previously been found in patients with PD. Our analysis shows that PARK5 is reduced 2-fold in PD. Variants of this protein have been associated with increases in α-synuclein levels in cultured cells. The ubiquitin-activating enzyme E1 transcript is also reduced in PD SN. These observations are consistent with a general pattern of accumulation of abnormal protein in PD and are probably not secondary effects; they were not detected in the PSP or FTDP samples.

We find a decrease in expression of 22 nuclear-encoded mitochondrial proteins, consistent with previous observation of decreases in complex I and complex IV activity in PD. This is unlikely to be secondary to reduced metabolic activity resulting from neuronal death: only 2 (COX4I1 and ATP1B1) of these 22 genes are also significantly reduced in PSP and FTDP, while 13 are elevated. This supports the recently postulated model of complex I dysfunction being the central player in initiating PD. The α-synuclein fly shows similar reductions in energy metabolism genes at early presymptomatic time points, although this trend is reversed later in the course of disease.

Intriguingly, PD (but not PSP or FTDP) patients express decreased levels of transcripts involved in protein trafficking, in general, and in neurotransmitter secretion, in particular. Vacular adenosine triphosphatases are involved in protein sorting and receptor-mediated endocytosis and have been directly implicated in neurotransmitter release. Eight different subunits of vacuolar adenosine triphosphatase are significantly underexpressed in PD SN compared with control specimens, correlating with the reduced expression of a novel lysosomal hydrogen adenosine triphosphatase seen in the α-synuclein fly. Neuronal exocytosis requires docking of multiple membrane proteins, such as synaptobrevin, which was reduced by more than 2-fold in PD SN. Even this small change could be biologically important, as synaptobrevin is normally present in stoichiometrically limiting amounts. The protein STXBP1 binds to syntaxin on the target membrane, forming part of the parallel 4-helix bundle that is thought to drive the fusion of opposing membranes. After membrane docking, calcium binds to synaptotagmin, triggering neurotransmitter release at the synapse. Our microarray analysis showed that expression levels of STXBP1 and synaptotagmin are significantly reduced in PD SN. This pathway is implicated in the Drosophila and yeast PD models: the A30P fly shows abnormal expression levels of lipid genes and the retinoid and fatty acid–binding glycoprotein gene (RFABG), and 18 of 57 genes implicated in the yeast PD model were clustered in the functionally related categories of lipid metabolism and vesicle-mediated transport.

Our microarray expression analysis of SN tissue from patients with PD identified candidate PD susceptibility genes and pathways, the importance of which is corroborated in PD model systems. We used expression analysis of the related neurodegenerative diseases PSP and FTDP to identify genes that may reflect secondary changes.

Table 3. Expression Changes in Parkinson Disease Model Systems

<table>
<thead>
<tr>
<th>Molecular Pathway</th>
<th>Human Substantia Nigra</th>
<th>Drosophila R406W MAPT</th>
<th>Drosophila A30P α-Synuclein</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaperones</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Ubiquitination</td>
<td>++++</td>
<td>. .</td>
<td>. .</td>
<td>++++</td>
</tr>
<tr>
<td>Vesicle trafficking</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>++++</td>
<td>. .</td>
<td>. .</td>
<td>++++</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>++++</td>
<td>. .</td>
<td>. .</td>
<td>++++</td>
</tr>
</tbody>
</table>

Abbreviations: MAPT, microtubule-associated protein tau gene; +, slightly involved; ++, moderately involved; ++++, very involved.

Figure. Cluster analysis. A, Venn diagram of genes used in cluster analysis. B, Supervised hierarchical cluster analysis with 238 genes (142 Parkinson disease [PD] vs control and 96 progressive supranuclear palsy and frontotemporal dementia with parkinsonism [PSP + FTDP] vs control). C, The same analysis excluding the 12 secondary effect genes.
Finally, we identified expression differences between PD, PSP, and FTDP that suggest a potential role for microarray analysis in future postmortem diagnostic procedures. Further studies with increased sample sizes and laser capture microdissection should provide further insight into this potential.

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Author Affiliations: Center for Human Genetics (Drs Hauser, Li, Noureddine, Shao, Stenger, and Vance, Messrs Xu, McLaurin, and Gibson, and Ms Jewett), Morris K. Udall Parkinson Disease Research Center of Excellence (Drs Hauser, Li, Noureddine, Scott, Hulette, and Vance, Messrs Xu, McLaurin, and Gibson, and Ms Jewett), and Departments of Pathology (Dr Hulette) and Medicine (Drs Hauser, Li, Schmechel, and Vance), Duke University, Durham, NC; Center for Neurologic Diseases and Morris K. Udall Parkinson Disease Research Center of Excellence, Harvard Medical School, Brigham and Women’s Hospital, Cambridge, Mass (Drs Gullans, Scherzer, and Jensen); and Department of Physics, Wesleyan University, Middletown, Conn (Dr Jensen).

Correspondence: Michael A. Hauser, PhD, Center for Human Genetics, Duke University, Duke University Medical Center 2903, Durham, NC 27710 (mike.hauser@duke.edu).

Author Contributions: Study concept and design: Hauser, Li, Gullans, and Vance. Acquisition of data: Hauser, Scherzer, Jensen, McLaurin, Gibson, Scott, Jewett, Schmechel, and Hulette. Analysis and interpretation of data: Hauser, Li, Xu, Shao, Noureddine, Gullans, Scherzer, Jensen, and Vance. Drafting of the manuscript: Hauser, Li, McLaurin, and Gibson. Critical revision of the manuscript for important intellectual content: Li, Xu, Noureddine, Shao, Gullans, Scherzer, Jensen, Scott, Jewett, Stenger, Schmechel, Hulette, and Vance. Statistical analysis: Li, Shao, Jensen. Obtained funding: Gullans, Hauser, and Vance. Administrative, technical, and material support: Hauser, Noureddine, Scherzer, McLaurin, Gullans, Jewett, Stenger, Schmechel, Hulette, and Vance. Study supervision: Hauser, Gullans, and Vance. Bioinformatics analysis: Xu.

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REFERENCES


