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

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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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erarchical 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.4

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.2 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.12 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.13

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 linkage2 (a table containing this supplemental information is available from

### Table 1. Tissue Donor Information

<table>
<thead>
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<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>87</td>
<td>M</td>
<td>III</td>
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<td>M</td>
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<tr>
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<td>58</td>
<td>F</td>
<td>NA</td>
<td>NA</td>
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</tr>
</tbody>
</table>

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

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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).

**COMMENT**

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-
sification analysis with more samples and appropriate cross-validation may be able to distinguish PD from PSP and FTDP.

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 previ-

ous 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 adeninosine triphosphatases are involved in protein sorting and receptor-mediated endocytosis and have been directly implicated in neurotransmitter release. Eight different subunits of vacuolar adeninosine triphosphatase are significantly underexpressed in PD SN compared with control specimens, correlating with the reduced expression of a novel lysosomal hydrogen adeninosine triphosphatase seen in the α-synuclein fly. Neuronal exocytosis requires docking of multiple membrane proteins, such as syntaptobrevin, which was reduced by more than 2-fold in PD SN. Even this small change could be biologically important, as syntaptobrevin 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 syntaptogamin, triggering neurotransmitter release at the synapse.

Our microarray analysis showed that expression levels of STXBP1 and syntaptogamin are significantly reduced in PD SN compared with control specimens. This pathway is implicated in the parallel 4-helix bundle that is thought to drive the fusion of opposing membranes. After membrane docking, calcium binds to syntaptogamin, triggering neurotransmitter release at the synapse. Our microarray analysis showed that expression levels of STXBP1 and syntaptogamin are significantly reduced in PD SN. This pathway is implicated in the yeast PD model: the A30P fly shows abnormal expression levels of lipid genes and the reti-
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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REFERENCES


