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Multiregional Gene Expression Profiling Identifies MRPS6 as a Possible Candidate Gene for Parkinson's Disease

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Combining large-scale gene expression approaches and bioinformatics may provide insights into the molecular variability of biological processes underlying neurodegeneration. To identify novel candidate genes and mechanisms, we conducted a multiregional gene expression analysis in postmortem brain. Gene arrays were performed utilizing Affymetrix HG U133 Plus 2.0 gene chips. Brain specimens from 21 different brain regions were taken from Parkinson's disease (PD) ($n = 22$) and normal aged ($n = 23$) brain donors. The rationale for conducting a multiregional survey of gene expression changes was based on the assumption that if a gene is changed in more than one brain region, it may be a higher probability candidate gene compared to genes that are changed in a single region. Although no gene was significantly changed in all of the 21 brain regions surveyed, we identified 11 candidate genes whose pattern of expression was regulated in at least 18 out of 21 regions. The expression of a gene encoding the mitochondria ribosomal protein S6 (MRPS6) had the highest combined mean fold change and topped the list of regulated genes. The analysis revealed other genes related to apoptosis, cell signaling, and cell cycle that may be of importance to disease pathophysiology. High throughput gene expression is an emerging technology for molecular target discovery in neurological and psychiatric disorders. The top gene reported here is the nuclear encoded MRPS6, a building block of the human mitoribosome of the oxidative phosphorylation system (OXPHOS). Impairments in mitochondrial OXPHOS have been linked to the pathogenesis of PD.

Key words: Parkinson's disease; Mitoribosome; MRPS6; Microarray; Postmortem

INTRODUCTION

Idiopathic Parkinson's disease (PD) is a multisystem disorder with a multifactorial etiology and diverse clinical phenotype. Only a small percentage (<5%) of patients develop PD that is linked to the currently known gene mutations (13). Significant advances in the understanding of the cellular and molecular pathways implicated in PD have been made by investigations focused on the function of five genes identified by linkage mapping. However, even with the current knowledge of these functional genomic pathways, it is likely that additional genes and gene products related to PD remain to be identified. Genome-wide association studies have demonstrated polymorphisms that confer susceptibility to PD (29).

Gene array surveys of the substantia nigra (SN) have provided additional insights into the biological, cellular, and molecular pathways implicated in PD (18,19,28,31). The only multiple region gene expression analysis suggested a number of novel disease mechanisms by narrowing the identified candidate gene list to include only those genes that were significantly regulated across all three brain regions (49). We have conducted to date the largest high-throughput gene array survey of cortical and subcortical brain

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regions ($N = 21$) in PD. Our working hypothesis was that if a gene is changed in more than one brain region, it may be a higher probability candidate gene compared to genes that are changed in a single region (34). We report here a list of differentially regulated genes and discuss their possible relevance to PD pathophysiology.

MATERIALS AND METHODS

Subjects and Biological Samples

Postmortem brain tissue was obtained from 21 brain areas in two groups of Caucasian subjects diagnosed with neuropathologically confirmed PD ($n = 22$) or aged individuals with no history or pathological diagnosis of neurologic or psychiatric disease ($n = 23$). All subjects consented during life to donate their brain after death to the University of Miami/NPF Brain Endowment Bank (UM/BEB). All subjects completed either a disease-specific (PD) or aged control registry form (normal, aged donors) providing information about demographics, clinical diagnosis, medications, environmental and drug and alcohol exposures, personal and family history, and activities of daily living. Yearly updates on all brain donors were obtained until death. Medical and hospital records were collected on an annual basis and all pertinent information was entered into a database. The clinical and pathological diagnosis of PD was based on the UK PD Society Brain Bank diagnostic criteria (22) and the severity of PD at death was assessed using the Hoehn and Yahr (H&Y) scale (21). All clinical records were reviewed by a movement disorders specialist (S.P.) to ensure that subjects met diagnostic criteria.

An agonal-state questionnaire (25 items) provided information about the events 48 h prior to death (time, date, place and cause of death, treating physician, mean 48-h axillary temperature, presence and type of infection, comorbidities, medication, presence of feeding tube, catheters, IV lines, PEG, oxygen, state of feeding and activity, and DNR status). This information was completed by the treating physician or nurse immediately after death and was used for exclusion of patients with prolonged agonal states or death-related events that are known to influence RNA quality (i.e., intubation or prolonged hypoxia). Although death certificates on all patients were available, they were not used as a source of information because they can introduce significant bias in PD (35). Because agonal state may affect the RNA expression profile of postmortem brain tissue, care was taken to match subject groups as closely as possible for age, gender, PMI, and brain pH. Regional samples of postmortem brain were taken from frozen coronal blocks based on surface and cytoarchitectural landmarks. The regional analysis included 21 different brain regions: substantia nigra, ventral tegmental area, perirhinal cortex (BA35), insular cortex, amygdala, nucleus basalis, caudate, putamen, nucleus accumbens, globus pallidus, mediodorsal thalamus, pulvinar, subthalamic nucleus, dorsal nucleus of the vagus nerve, cerebellar hemisphere, anterior cerebellar vermis, dorsal raphe, locus ceruleus, hypothalamus, hippocampus and reticular formation.

Microarray Experiments

Total RNA isolation and biotin-labeled cRNA synthesis were performed by Gene Logic Inc. (Gaithersburg, MD) using a TriZol method and RNEasy columns, according to Affymetrix (Santa Clara, CA) specifications from 50 mg of each regional sample. Extractions of RNA used in the present study had a minimum A260/A280 ratio of more than 1.9. The samples were further checked for evidence of degradation and integrity. Samples had a minimum 28S/18S ratio of more than 1.6 (2100 Bioanalyzer; Agilent Technologies, Palo Alto, CA). We used the Human Genome U133 Plus 2.0 GeneChip array with 54,000 probe sets representing more than 47,000 transcripts derived from approximately 38,500 well-substantiated human genes (available at: http://www.affymetrix.com). Gene chip analysis was performed with Microarray Analysis Suite version 5.0, Data Mining Tool 2.0, and Microarray database software (available at: http://www.affymetrix.com). The genes represented on the gene chip were globally normalized and scaled to a signal intensity of 100.

The different measures of microarray RNA integrity are shown for the insula, caudate, and the substantia nigra in Table 1. The same values were compared in all 21 regions to filter samples for quality control to meet criteria for inclusion in the final analysis. Microarray quality control parameters included the following: noise (RawQ), consistent number of genes detected as present across arrays, consistent scale factors, and consistent β-actin and glyceraldehyde-3-phosphate dehydrogenase 5′/3′ signal ratios.

Data Analysis

We performed a gene expression survey for each of the 21 individual regions comparing end-stage PD patients and normal aged subjects. The fold-change values from each subject were averaged across regional samples. From a total of 945 samples obtained from the 21 brain regions from PD brain donors ($n =$

TABLE 1
BRAIN SAMPLE MICROARRAY QUALITY CONTROL PARAMETERS

Region	Sample No.	Age (Years)	β-Actin Ratio	GAPDH Ratio	RNA QC	Present Calls	% Present	RAWQ	Scale Factor	PMI
Substantia nigra										
CTRL	11	77.9 ± 13.09	0.33 ± 0.11	0.60 ± 0.14	0.46 ± 0.11	24296 ± 753.24	44.4 ± 7.8	1.53 ± 0.08	2.00 ± 0.24	8.2 ± 2.1
PD	16	75.1 ± 7.81	0.28 ± 0.09	0.58 ± 0.11	0.42 ± 0.09	24982 ± 837.18	45.5 ± 1.50	1.60 ± 0.23	1.85 ± 0.26	7.4 ± 1.6
Insula										
CTRL	11	73.3 ± 4.02	0.35 ± 0.04	0.62 ± 0.04	0.48 ± 0.04	25633.9 ± 360.5	46.8 ± 6.8	1.74 ± 0.05	1.45 ± 0.07	9.3 ± 2.0
PD	12	72.2 ± 3.13	0.35 ± 0.03	0.66 ± 0.04	0.50 ± 0.03	24892.5 ± 582.5	45.6 ± 1.2	1.53 ± 0.14	2.03 ± 0.29	7.5 ± 1.8
Caudate										
CTRL	14	71 ± 6.9	0.27 ± 0.06	0.58 ± 0.06	0.43 ± 0.05	23927 ± 585.8	43.8 ± 10.8	1.55 ± 0.06	2.05 ± 0.16	9.8 ± 2.7
PD	10	73 ± 3.07	0.31 ± 0.02	0.625 ± 0.047	0.45 ± 0.04	26259.4 ± 337.2	48.10 ± 6.6	1.61 ± 0.06	1.61 ± 0.13	8.9 ± 1.8

CTRL, normal aged control subjects; PD, Parkinson's disease subjects.

22) and normal aged controls ($n = 23$), a total of 499 (52.8%) passed the brain microarray quality control and were used in the final expression data analysis. The total number of samples per region per subject is presented as a tiling chart and is shown in Figure 1. We selected genes for analysis on the basis of present calls by Microarray Analysis Suite 5.0. In the present study, for a gene to be included, it had to be present (detectable) in at least 75% of the subjects to reduce the chances of false-positive findings. Expression data were analyzed using Genesis (GeneLogic, Gaithersburg, MD) and AVADIS software (Strand Genomics, Redwood City, CA). Gene expression values were floored to 1 and then $\log_2$-transformed. One-way analysis of variance was performed for each gene to identify statistically significant gene expression changes. Two criteria were used to determine whether a gene was differentially expressed. The cutoffs for inclusion were a one-way analysis of variance value of $p \leq 0.05$ and a fold-change (FC) of ±1.3. An additional analysis restricted to a comparison of affected and control male subjects was performed in the substantia nigra and perirhinal cortex to rule out the possible confounding effects of gender on the analysis.

Target Validation

All cases included in the microarray analysis (PD $n = 22$ and aged control $n = 23$) were used for the quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) for selected target validation. For validation of the MRPS6 gene, we selected two out of 21 regions (caudate and insula) for real-time RT-PCR experiments. Three housekeeping genes [cyclophilin, β-actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)] were used as endogenous controls to generate a normalization factor for quantitative comparisons across groups of PD patients and aged controls.

Total RNA was isolated using the TriZol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The concentration of RNA was determined by spectrophotometry, using GeneQuant

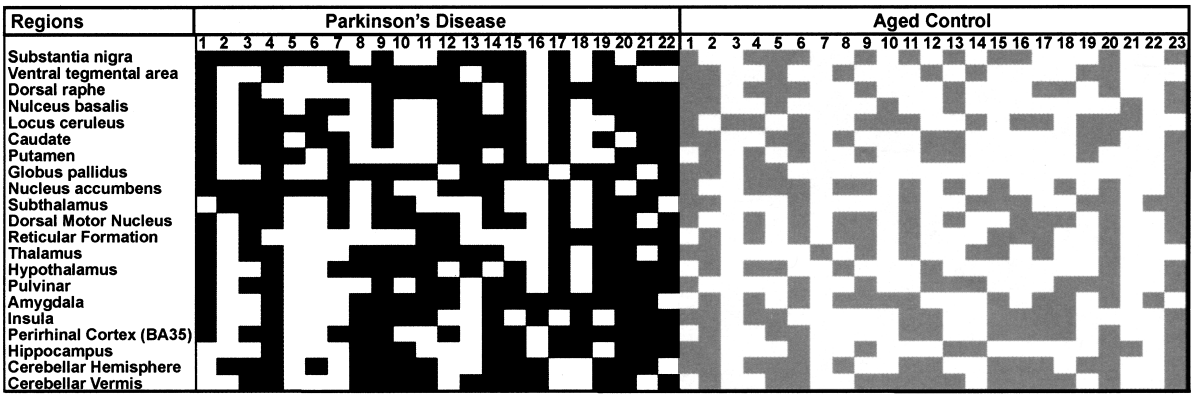

Figure 1. The regional brain sample tiling chart. Columns represent gene chips from individual subjects and rows illustrate gene chips per regional comparison. Data from a total of 499 gene chips (283 from 22 PD patients and 216 from 23 aged control subjects) represent a sample from 21 regions that passed both the RNA and microarray quality control.

II (Amersham Biosciences, Uppsala, Sweden). Reverse transcription was performed with SuperScript™ First-Strand Synthesis System (Invitrogen). Real-time PCR reactions were run in 96-well PCR plates using an ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA). Each 50-µl reaction contained cDNA template generated from RNA, 900 nM of gene-specific primers for MRPS6 (5′-AT GGGATCTCTGCCC CAGTCA-3′ and 5′-CAAGT GCTCACCATGCTTT-3′), 250 nM probe (5′-FAM TTTTTATGCACCCACCGCAGC-3′), and Taqman Universal PCR Master Mix (Applied Biosystems) containing Hot Goldstar DNA Polymerase, dNTPs, uracil-*N*-glycosylase, and passive reference. PCR cycle was run at 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. At the end of PCR cycling steps, data were collected by the Sequence Detector Software (SDS version 2.1, Applied Biosystems). All measurements were performed in triplicate and the gene expression levels calculated as an average of triplicates. To normalize the intersample variation in RNA, three housekeeping genes (cyclophilin, β-actin, and GAPDH) were quantified for all samples. Target primers and probe mix to detect target selection were done according to manufacturer's specification (Applied Biosystems). A normalization factor calculation based on the geometric mean was derived using geNorm [Visual Basic Application (VBA) for MS Excel] (43).

RESULTS

The RNA quality parameters for all subjects are shown in Table 1 for two blindly selected regions (insula and caudate also used for real-time RT-PCR validation) and the substantia nigra. Analysis of the quality control parameters showed no significant differences in age, gender, brain pH, postmortem interval, or RNA QC values between aged control and PD groups. Table 2 lists the demographic information, age at death, cause of death, PMI, and brain pH values for PD and control subjects. These results demonstrate that the subjects were well matched on these variables, including the number of individuals with sudden versus prolonged terminal cause of death. The clinical characteristics of the PD subjects included in the gene expression survey are summarized in Table 3. All PD subjects had advanced disease with a mean H&Y stage of 4.5 ± 0.7. Consistent with previous reports, RNA quality control parameters showed no effect of PMI even in a brain region (substantia nigra) that is one of the most severely affected with advanced disease (Fig. 2).

A presentation of lists of hundreds of differentially expressed genes derived from region-by-region comparisons ($n = 21$) of postmortem brain tissue from PD patients and aged controls was beyond the scope of this study. To reduce the number of false positives, we conducted a multiregional survey to identify and cluster select genes that were differentially expressed across the different comparison regions. A frequency pyramid indicating the number of target brain regions in which these candidate genes were significantly expressed is shown in Figure 3. A list of these top 11 genes associated with PD in the multiregional comparison is presented (Table 4), together with biological function and chromosomal location (Table 5). The differential pattern of up- or downregulation for each gene was consistent across all brain regions. Topping the list was MRPS6, a nuclear encoded mitochondrial ribosomal protein, that was significantly upregulated with a 2.1 mean fold change for 20 out of 21 regions ($p < 0.001$). The only brain region in which the MRPS6 was not differentially expressed was the hippocampus. We confirmed that analysis was unbiased by gender by restricting the inclusion of brain regional samples to male subjects. This subgroup analysis gave the same pattern of differentially expressed genes across regions in male subjects (data not shown). PRKACB and FUSIP1 both map to chromosomal locus (1p36), which has three PD loci: PARK6 (pink1) (42), PARK7 (dj1) (4), and PARK9 (unknown gene) (45).

Gene Ontology (GO) analysis of the top candidate genes identified in the multiregional analysis suggests involvement of genes that respond to stress in end-stage disease, including STIP1 and CIRBP (Tables 4 and 5). Another gene of interest was the solute carrier family member 2 (SLC38A2), which functions to transport glutamine. This gene product was upregulated 1.6-fold in PD compared to control subjects. We observed in 18 of the 21 PD brain regions surveyed, a marked approximately twofold decrease for the cAMP-dependent protein kinase, beta catalytic subunit (PRKACB). The gene has been implicated in a number of different cellular processes, including cell growth and death and long-term potentiation in the CNS.

The expression levels of MRPS6 were confirmed by quantitative real-time RT-PCR analyses in two blindly selected regions (caudate nucleus and insula). The validation results are presented in Figure 4. We used three control genes (GAPDH, β-actin, and cyclophilin) to normalize expression data for MRPS6. The correlation between the microarray and real-time RT-PCR data using the three controls genes for real-time RT-PCR were consistent for this gene product. Analysis of the MRPS6 gene in PD patients compared to normal aged controls was of the same order of magnitude as seen from the microarray experi-

TABLE 2
DEMOGRAPHIC DETAILS, CAUSE OF DEATH, AND RNA QUALITY DETERMINING PARAMETERS

Code	Gender	Age at Death	Cause of Death	PMI	pH
Control*					
C1	M	74	Lung cancer	4	6.52
C2	F	90	Congestive heart failure	5	6.08
C3	F	90	Respiratory failure	5	6.12
C4	F	83	Chronic renal failure	13	6.52
C5	F	85	Metastatic cancer of lung	3	6.04
C6	M	46	Myocardial infarction	7	6.5
C7	F	85	Obstructive pulmonary disease	15	6.53
C8	F	82	Malignant melanoma	5	6.92
C9	F	84	Cardiorespiratory arrest	4	6.01
C10	F	90	Heart and respiratory failure	3	6
C11	M	88	Multiple organ failure	3	6.03
C12	F	84	Cerebrovascular accident	9	5.96
C13	M	85	Myelodisplastic syndrome	11	6.06
C14	M	80	Myelodisplastic syndrome	12	6.08
C15	F	85	Respiratory failure	4	6.3
C16	F	83	Cardiopulmonary arrest	4	6.16
C17	F	88	Ischemic heart disease	11	5.93
C18	M	65	Ischemic heart diseae	9	6.64
C19	M	70	Cardiac arrest	9	6.18
C20	M	65	Ischemic heart disease	6	6.49
C21	M	65	Heart disease	10	6.45
C22	M	65	Cardiac arrest	12	6.78
C23	M	68	Cardiac arrest	16	6
Parkinson's disease†					
PD1	F	65	Cardiopulmonary failure	4	6.28
PD2	M	75	Infectious disease/IHD	15	6.36
PD3	M	77	PD	4	6.38
PD4	M	71	Ischemic heart diseae	6	6.00
PD5	M	74	PD/Ischemic heart diseae	4	6.41
PD6	M	63	Cardiopulmonary failure/PD	5	6.42
PD7	M	88	Aspiration/PD	20	6.48
PD8	M	66	PD	5	6.42
PD9	F	86	Cardiopulmonary failure	5	6.59
PD10	M	78	PD	5	6.16
PD11	M	71	Intestinal bleeding	12	6.05
PD12	F	60	Stroke	4	6.29
PD13	F	66	Respiratory failure	10	6.51
PD14	M	83	PD	2	5.96
PD15	M	74	PD	8	6.42
PD16	M	72	Pneumonia	6	5.88
PD17	M	69	PD/cancer	3	6.00
PD18	M	82	Cardiopulmonary failure	4	5.79
PD19	M	73	Coronary artery disease	11	5.92
PD20	M	76	Pneumonia/PD	5	5.97
PD21	M	81	Coronary artery disease	5	6.02
PD22	F	78	Coronary artery disease/PD	6	6.39

*Total 23: 11 male, 13 female; age at death 78.2 ± 11.4; 10 suden death, 13 prolonged; PMI 7.8 ± 4.1; pH 6.3 ± 0.3.
†Total 22: 17 male, 5 female; age at death 74 ± 7.4; 11 sudden death, 12 prolonged; PMI 6.8 ± 4.8; pH 6.2 ± 0.2.

TABLE 3
CLINICAL CHARACTERISTICS OF PARKINSON'S DISEASE SUBJECTS

Code	Gender	Age at Onset	Disease Duration	H&Y	Onset Sympton	Dementia	Depression	Dyskinesia	Autonomic Dysfunction
PD1	F	50	15	4	tremor	no	no	yes	no
PD2	M	67	8	5	gait	no	no	no	no
PD3	M	64	13	5	gait	no	no	yes	no
PD4	M	51	20	5	slowness	no	no	yes	no
PD5	M	53	21	5	tremor	no	no	yes	syncope
PD6	M	53	10	5	tremor	no	yes	no	no
PD7	M	77	11	4	tremor	no	no	no	no
PD8	M	55	11	5	tremor	no	no	yes	no
PD9	F	77	9	5	tremor	no	yes	no	no
PD10	M	56	22	5	gait	yes	yes	yes	no
PD11	M	44	27	5	tremor	no	no	yes	no
PD12	F	40	20	3	stiffness	no	no	yes	incontinence
PD13	F	56	10	5	tremor	yes	yes	no	no
PD14	M	80	3	4	tremor	no	yes	no	hypotension
PD15	M	64	10	4	stiffness	yes	yes	yes	constipation
PD16	M	46	26	4	stiffness	yes	yes	yes	hypotension
PD17	M	59	10	5	tremor	no	no	yes	no
PD18	M	74	8	3	depression	no	yes	no	no
PD19	M	70	3	3	gait	yes	yes	no	incontinence
PD20	M	72	4	5	gait	yes	no	no	incontinence
PD21	M	68	13	5	tremor	yes	no	yes	incontinence
PD22	F	68	10	5	gait	yes	no	yes	no

H&Y, Hoehn and Yahr clinical stage of Parkinson's disease (1 = mild unilateral disease to 5 = severe end-stage disease). Seventeen males and 5 females; age at onset 61.1 ± 11.5; disease duration 12.9 ± 6.9; HY 4.5 ± 0.7.

ments. The fold change values determined from microarray (caudate FC = 2.7 ± 0.1 and insula FC = 2.7 ± 0.1) were in good agreement with expression levels determined by real-tim RT-PCR (caudate FC = 2.6 ± 0.3 and insula FC = 2.4 ± 0.3) (Fig. 4). These results demonstrate that the differential expression of MRPS6 was confirmed by both methods on samples from the same individuals.

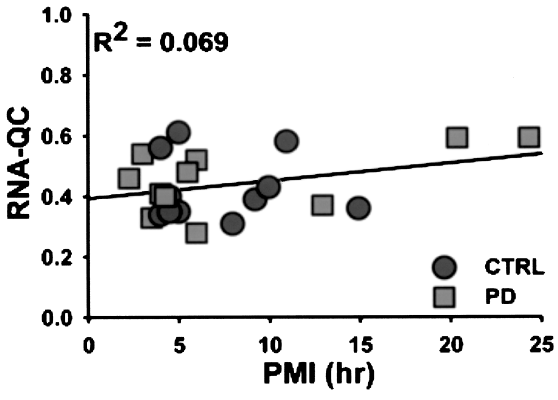

Figure 2. RNA quality control (QC) parameters for the substantia nigra. The relationships between RNA QC (determined by the average of the 5′/3′ signal ratios of β-actin and GADPH across Plus 2.0 chips) and the postmortem interval (PMI) in SN show no effect of the PMI on the QC measurements.

DISCUSSION

Gene expression profiling is done usually on only a few select brain regions, providing a measurement of transcript numbers at a particular point in the sequence of a continuing process. The expression levels of specific gene transcripts in postmortem brain tissues may be either "state" or "trait" dependent, reflecting a complex interplay of disease-relevant changes in cellular processes, structure, and function. This report provides the first extensive multiregional gene expression profiling survey in PD, to identify

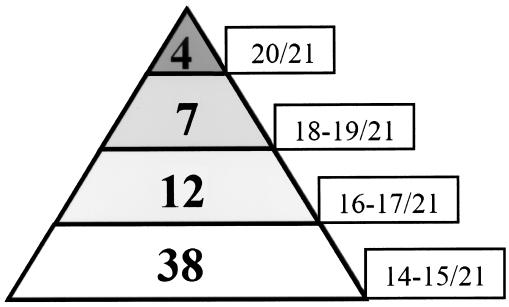

Figure 3. Frequency pyramid illustrates the number of candidate genes differentially expressed in PD versus normal aged control subjects. Four genes topped the pyramid (20 out of 21 regions surveyed).

TABLE 4
SUMMARY OF THE GENES IDENTIFIED AS DIFFERENTIALLY EXPRESSED IN AT LEAST 18 OUT OF 21 REGIONS

Gene Symbol	Affymetrix Gene Fragment ID*	Gene Name	No. of Regions	Regions Not Present	Mean FC	Mean *p*
MRPS6	212944_at	Mitochondrial ribosomal protein S6	20	HIPP	2.1	0.001
HIST1H2BD	235456_at	Histone 1, H2bd	20	Pt	2.0	0.012
RBM3	208319_s_at	RNA binding motif (RNP1, RRM) protein 3	20	LC	−1.4	0.006
SLC38A2	222982_x_at	Solute carrier family 38, member 2	20	HIPP	1.6	0.006
CHORDC1	218566_s_at	Cysteine/histidine-rich domain (CHORD)-containing, zinc binding protein 1	19	HIPP, Pulv	1.9	0.007
CIRBP	200810_s_at	Cold inducible RNAS binding protein	19	HIPP, Pulv	−2.0	0.007
FLJ33814		Hypothetical protein FLJ33814	18	AGM, Cere	1.5	0.006
FUSIP1	225348_at	FUS interacting protein (serine/arginine-rich) 1	18	HIPP, LC, VTA	1.6	0.012
PRKACB	225644_at	Protein kinase, cAMP-dependent, catalytic, beta	18	HIPP, NB, Th	−1.9	0.007
STIP1	213330_s_at	Stress-induced phosphoprotein 1 (Hsp 70/Hsp 90-organizing protein)	18	AMG, Pulv, Th	1.8	0.015
SUV420H1	222759_at	Suppressor of variegation 4-20 homolog 1 (Drosophila)	18	NB, Pulv, Th	−1.6	0.012

HIPP, hippocampus; Pt, putamen; LC, locus ceruleus; Hyp, hypothalamus; Pulv, Pulvinar; AMG, amygdala; Cere, cerebellar hemisphere; VTA, ventral tegmental area; NB, nucleus basalis, Th, thalamus.
*Annotated from www.affymetrix.com

common transcripts that are regulated reproducibly throughout a large number of the total number of brain regions surveyed.

Using this approach, we have identified MRPS6 as a top gene associated with PD. MRPS6 was significantly upregulated in 20 out of 21 regions studied, approximately twofold that of normal aged controls. The expression levels of MRPS6 in PD were confirmed by quantitative real-time RT-PCR. A total of three other high probability genes were differentially expressed in addition to the MRPS6, including the histone 1 (HIST1H2BD), RNA binding motif protein 3 (RBM3), and solute carrier family 38, member 2 (SLC38A2), a sodium-coupled glutamine transporter (41) in 20 out of 21 regions surveyed. Other genes of interest that were regulated throughout most of the brain regions surveyed were the cysteine and histidine-rich containing zinc binding protein 1 (CHORDC1), the cold inducible RNA binding protein (CIRBP), a heat shock protein 90 (HSP90)-interacting protein (46), cAMP-dependent protein kinase, beta catalytic subunit (PKBACB), and a stress-induced phosphoprotein (STIP1).

Mitochondrial dysfunction caused either by genetic defects (i.e., PINK1 and DJ1 mutations) and/or environmental factors (MPTP, rotenone or paraquat toxicity) causes parkinsonism in vivo in mice and primate models (13,14). PD is a multisystem disorder that affects autonomic, limbic, and somatomotor systems with advanced disease staging. Our finding of increased expression of MRPS6 in PD patients may be associated with a disorder of energy metabolism with development of PD-related pathology. In the first reported high-resolution whole-genome association study of PD, single nucleotide polymorphism (SNP) analysis revealed disease associated SNPs within a gene designated 12 LOC200008, which encodes a hypothetical protein with inferred oxidoreductase activity and potential involvement in cholesterol biosynthesis and electron transport (29). Interestingly, these SNPs are also within 21 kb upstream of the mitochondrial ribosomal protein gene (MRPL37).

Nuclear MRP genes are associated with mitochondrial disease (17,33). It is estimated that there are about 100 different human mitochondrial ribosomal proteins (32), all of which are encoded by nuclear genes (33). They are essential building blocks for the 55S mammalian mitochondrial ribosome, which translates mitochondrial mRNAs for the 13 essential components of the OXPHOS (33). The mammalian mitoribosome differs significantly from the ancestral 70S ribosome (17) in that it has lost nearly half the RNA present in bacterial mitoribosomes and gained "extra" proteins (MRPs) (37), which can have additional properties (multifunctional) and have been implicated, among others, in apoptosis and cellular degeneration (8).

Because mitochondrial ribosomes are responsible for translating the 13 mRNAs for essential proteins of the OXPHOS, mutations in these proteins have significant consequences. Several of the MRP genes map to chromosomal loci associated with neurological/neurodegenerative diseases (33). These range from mild, late-onset disorders, such as age-related sensorineural hearing impairment or ocular myopathy (PEO), to devastating and usually fatal infantile disorders, such as Leigh syndrome (also known as fatal necrotizing encephalopathy) (23). MRPs have been

TABLE 5
TOP GENES, BIOLOGICAL FUNCTION, GENE ONTOLOGY, AND CHROMOSOMAL LOCATION

Gene Symbol	Biological Function	Gene Ontology*		Location
		Process	Component	
MRPS6	Structural constituent of ribosome	Protein biosynthesis	Mitochondrial small ribosomal subunit	21q21.3-q22.1
HIST1H2BD	DNA binding	Chromosome organization and biogenesis, nucleosome assembly	Nucleus, chromosome, nucleosome	6p21.3
RBM3	RNA processing	RNA, nucleotide, nucleic acid binding	N/A	Xp11.2
SLC38A2	A sodium-coupled neutral amino acid transporter (16)†	Glutamate–glutamine cycle/gultamine transporter (16)†	N/A	12q
CHORDC1	Heat shock protein 90-interacting protein (17)†	N/A	N/a	11q14.3
CIRBP	RNA, nucleotide and nucleic acid binding	Response to cold	Nucleus	19p13.3
FLJ33814	N/A	N/A	N/A	22q12.1
FUSIP1	RNA splicing factor activity, transesterification mechanism, RS domain binding, unfolded protein binding	Assembly of spliceosomal tri-snRNP, cytoplasmic transport, mRNA export from nucleus, mRNA splice site selection, nuclear mRNA splicing, via spliceosome, regulation of transcription	Cytoplasm, nucleoplasm, nucleus	1p36.11
PRKACB	ATP binding, cAMP-dependent protein kinase activity, magnesium ion binding, nucleotide binding, protein serine/threonine kinase activity, transferase activity	G-protein signaling, coupled to cAMP nucleotide second messenger, protein amino acid phosphorylation, signal transduction	cAMP-dependent protein kinase complex, nucleus	1p36.1
STIP1	Binding	Response to stress	Golgi apparatus, nucleus	11q13
SUV420H1	Histone lysine *N*-methyltransfease	Histone methylation	Condensed nuclear chromosome, pericentric region	11q13.2

N/A, not assigned.
*Annotated from Entrez Gene (www.ncbi.nlm.nih.gov).
†Proposed biological function (see reference).
‡Drosophila analogue.

linked also to diseases affecting specific neuronal populations including nonsyndromic hearing loss (40), spinocerebellar ataxia with blindness and deafness (6p23-p21) (33), Usher syndrome, type 1E (21q21) (10), Leigh syndrome (9q34, 11q13, 19p13.3, 5q11) (12), Russell-Silver syndrome (7p11.2, 17q23-q24) (30), the Stuve-Wiedemann syndrome (1p34) (9), and the multiple mitochondrial dysfunctions syndrome (2p14-p13) (38).

Mitochondrial dysfunction plays a key role in many signaling pathways leading to cell death (6,16). The precise mechanisms underlying the role of mitochondria in apoptosis (2) and the number of proteins involved remain unclear (11). MRPS29 shares sequence homology with death-associated protein 3 (DAP3) (7,25,26,39). DAP3 is a GTP binding protein that mediates interferon-, tumor necrosis factor-, and FAS-induced cell death (8). When overexpressed, it causes apoptosis in a number of different types of mammalian cells (25,26). Although its precise role in the induction of cell death is not known, it functions downstream of the death-inducing signaling complex, but upstream of some members of the caspase family (25,26). A second proapoptotic protein of unknown function is programmed cell death protein 9 (PDCD9 or p52) (designated MRP-S30) (8). Yoo et al. (47) demonstrated that the mitoribosomal protein MRPL41 enhances p53 stability and contributes to p53-induced apoptosis in response to growth-inhibitory conditions. The tumor suppressor p53 is a key regulator of both the cell cycle and cell proliferation (47). The p53 protein is a potent transcription factor that activates target genes and triggers growth arrest, DNA repair, or apoptosis in response to cellular genotoxic stresses (15,24). MRPL41 protein enhances the translocation of p53 to the mitochondria, thereby

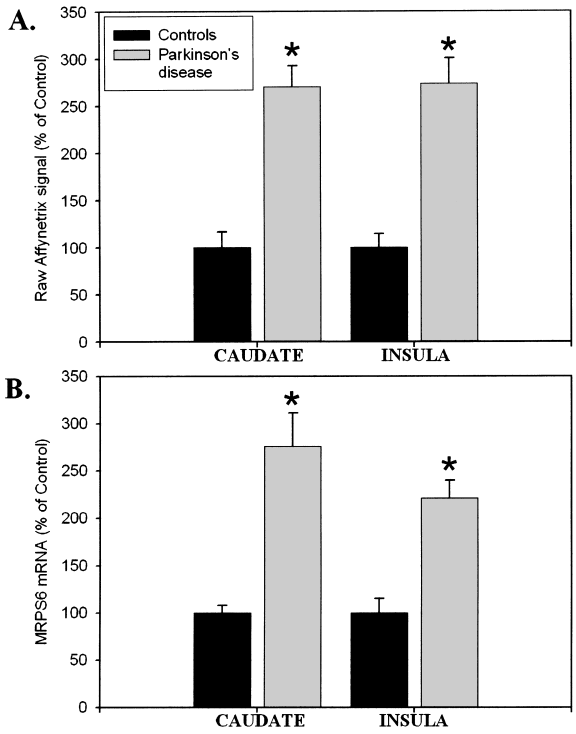

Figure 4. Comparison of MRPS6 gene expression in PD patients versus normal aged controls in selected regions: (A) Raw Affymetrix data show significant upregulation of MRPS6 gene expression in the caudate and the insula. (B) Quantitative real-time RT-PCR gave comparable results of MRPS6 expression in both regions. *$p \leq 0.001$.

inducing apoptosis. While the function of MRPS6 is at present unknown, the link to proapoptotic mechanisms demonstrated for other members of the mitoribosomal family makes this protein a potentially relevant gene target for PD pathophysiology.

The expression of the β catalytic subunit of cAMP-dependent protein kinase (PRKACB) gene was significantly downregulated in our multiregional comparisons between PD subjects and normal, aged controls. PRKACB regulates the function of the neurotrophin receptor p75 by phosphorylation (20). Several p75 neurotrophin receptor-mediated activities have been proposed, including enhancement of axonal outgrowth (1,5) and modulation of dopaminergic synaptic transmission (3). A recent report suggests metaplasticity of the late phase of long-term potentiation includes a critical role for cAMP/protein kinase A signaling (48). A disruption in this pathway in advanced PD might suggest a loss of input-specific synaptic facilitation and relative imbalance due to loss of protein kinase A activity.

Another gene of interest identified in our study is the solute carrier family 38, member 2 (SLC38A2), a sodium-coupled glutamine transporter. SLC38A2 is thought to be involved in the glutamate–glutamine cycle (41). Glutamate released into the extracellular space is cleared by glutamate transporters (expressed in neuronal cells and glial cells), terminating neurotransmission. Glutamate is converted to glutamine by glutamine synthetase (36) and then rereleased through glutamine transporters, like SLC38A2) (27) for uptake by glutaminergic neurons as an immediate precursor of glutamate (41). Interestingly, SLC38A2 maps to the same region as PARK8 (LRRK2) (50). The upregulation of SLC38A2 may link this transporter with a dysregulation of glutaminergic pathways, consistent with the excitotoxic theory of neurodegeneration proposed for PD (44).

Gene expression analysis is important for understanding complex patterns of transcript regulation that are relevant for identification of genes implicated in PD. Despite the importance of this rapidly advancing technology, the interpretation is limited by a high rate of false positives. However, we point out here that the use of a multiregional comparison limits this possibility in our study. The major strength of this study relates to its sample size. cDNA samples were synthesized from 45 total patients producing 499 useable samples, all of which were subjected to gene chip hybridization and analysis. Such scaling significantly reduces the false discovery rate, which can be sizeable when screening the transcriptional activity of the human genome. Also, the strict inclusion criteria employed, the careful case selection, and RNA quality control parameters provide additional assurances that the top candidates identified in our study are potentially relevant targets that warrant further study. The results shown for MRPS6 were based on consistency between different gene probe signals across brain regions and validated using an alternative method (real-time RT-PCR).

An association of an overexpression of the MRPS6 gene with PD requires replication across larger cohorts to verify disease specificity. Independent replication, protein validation and convergenent functional genomic approaches are needed to validate the proposed association of this novel MRP with impaired energy metabolism and cell death in PD.

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