Gene Expression Responses in a Cellular Model of Parkinson's Disease

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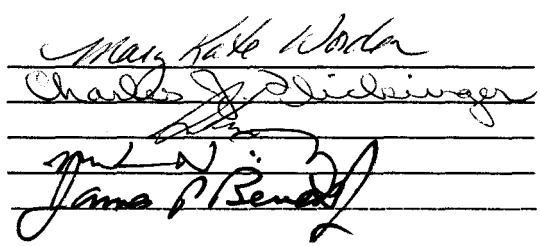

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Abstract

This research represents initial steps towards understanding the relation between changes in gene expression, mitochondrial function and cell death in cell-based models of Parkinson's disease. The main hypothesis is that rapid gene expression changes in cells exposed to parkinsonian neurotoxins occur, are dependent on mitochondrial status, and directly impact intracellular signaling pathways that determine whether a cell lives or dies. Our cellular model is comprised of SH-SY5Y neuroblastoma cells exposed to the parkinsonian neurotoxin methylpyridinium ion. Transcriptomic changes are evaluated with nylon and glass-based cDNA microarray technology. Cardinal symptoms of Parkinson's disease, characteristic pathological changes, therapeutic modalities, and current theories on the etiology of the disorder are discussed. Our results verify the existence of mitochondrial-nuclear signaling in the context of electron transport chain deficits, as well as suggesting the vital roles played in this process by previously described intracellular signaling pathways. These results will serve to direct future investigations into gene expression changes relevant to the processes of cell death and cell survival in our cellular model of Parkinson's disease, and may provide important insights into the pathophysiology of the in vivo disease process.

Chapter 1

My research in the Center for the Study of Neurodegenerative Disease has explored the relation between changes in gene expression, mitochondrial function and cell death in cell-based models of Parkinson's disease. The main hypothesis behind my work is that rapid gene expression changes in cells exposed to parkinsonian neurotoxins occur, are dependent on mitochondrial status, and directly impact intracellular signaling pathways that determine whether a cell lives or dies. I have attempted to bring two novel experimental techniques to bear upon this problem: cDNA microarrays and RNA interference (RNAi). This introductory chapter will serve as a review of the background information necessary to place the dissertation research in its proper context. The cardinal symptoms and pathology of Parkinson's disease will be discussed, as will the primary therapeutic modalities, and major hypotheses regarding the etiology of the disorder. The most recent developments in cDNA microarrays and RNA interference (RNAi) will be reviewed. Subsequent chapters will document my experimental techniques and results, along with my suggestions for future directions in cellular and molecular Parkinson's disease research.

Background Information

Parkinson's Disease

Parkinson's disease is a progressive neurodegenerative disorder that can cause profound motor impairment in its victims. It is among the most prevalent neurological disorders, with approximately 50,000 new cases diagnosed each year in the United States alone. The four cardinal symptoms of PD are bradykinesia, tremor at rest, postural instability, and rigidity. Tremor is the symptom that is often first to prompt a patient to seek medical attention; the tremor of PD is described as a "pill-rolling" tremor, referring to the stereotyped motion of the fingers and hands. It is of low frequency (about 4-5 cycles per second) and occurs at rest. The characteristic Parkinsonian tremor tends to reduce in severity or even disappear when the patient initiates a voluntary movement.

Bradykinesia, a slowing of movements in general, is often present early in the clinical course of PD and can become highly debilitating. Inability to execute sequential motor tasks as a result of bradykinesia frequently has severe impacts on the patient's ability to perform activities of daily living. *Rigidity* is generally manifested in movements of the extremities (cogwheel rigidity) and, in moderate to severe cases of PD, episodes of "freezing," or the inability to initiate any voluntary movements. In addition to the obvious reduction in ability to perform activities of daily living, such episodes often provoke intense feelings of fear and helplessness, with negative impacts on the patient's mental and emotional well-

being. Some reports have also detailed the phenomenon of *akinesia paradoxica*, referring to temporary periods of normal or near normal motor activity under stressful situations.

Postural abnormalities manifest in the PD patient with unusual degrees of flexion in the limbs. The trunk is often stooped or hunched forward, and when combined with flexion of the knees and elbows, the patient's center of gravity is frequently displaced forward to a sufficient degree to cause the characteristic shuffling gait of PD. As the disease progresses, cerebellar reflexes required to maintain balance are lost, with the frequent onset of episodes of falling. Falls are a major cause of morbidity and mortality in PD patients, as for the elderly population at large.

Other non-motor symptoms frequently associated with the disease process, particularly in its early stages, include a generalized loss of manual dexterity, manifesting as difficulty in performing simple motor tasks, mild depression, a reduction of facial expressions (mask facies) and *micrographia*, a reduction in size of handwriting. On the order of forty percent of PD patients present with non-specific sensory complaints, including numbness, tingling, aching or burning sensations, particularly in the extremities. A high percentage of patients present with *anosmia*, loss of the sense of smell. Anosmia may occur years before onset of the cardinal motor symptoms of PD. As the olfactory tissues are directly exposed to the environment, this phenomenon has been invoked by some researchers as an endorsement of the environmental hypothesis of PD, i.e., that a neurotoxic agent responsible for PD present in the

environment either gains entry to the brain via the olfactory nerves, or that the olfactory receptors alternatively represent a highly vulnerable neuronal population whose demise is linked to chronic exposure to a Parkinsonian neurotoxin.

Dementia is frequently reported in PD, but there exists considerable variability in reports of its rate of occurrence (10-40%). Some investigators have reported a higher prevalence of dementia in PD in males, and in those patients of more advanced age. Likewise, depression is reported in a variable but high portion of the PD population (37-90%). As depression often occurs early in the clinical course, It is unclear whether the majority of depressive symptoms are related to the etiology of PD, or rather a reaction to symptoms on the part of the patient.

Historical Context

Parkinson's Disease (PD) is generally referred to as the archetype of the hypokinetic movement disorders. The symptoms of this condition were first described in the 1817 "Essay on the Shaking Palsy" by James Parkinson, a general practitioner in the suburbs of London (Parkinson 1817). The impetus for the essay was Parkinson's keen observations of Londoners with characteristic gait abnormalties, tremors and muscle weakness that tended to increase in severity with advancing age:

"The patient can [rarely] form any recollection of the precise period of its commencement. The first symptoms perceived are, a slight sense of weakness, with a proneness to trembling in some particular part; sometimes in the head, but most commonly in one of the hands and arms…. The propensity to lean forward becomes invincible…. As the debility increases and the influence of the will over the muscles fades away, the tremulous agitation becomes more vehement."

Parkinson and his contemporaries were hamstrung by the lack of a standardized system of neurological examination, a problem that continues in part to the present day. PD remains a diagnosis that is based primarily on clinical observation by the physician, with autopsy examinations of brain tissue and quantitative assessments of symptoms playing secondary roles. It was not until half a century following the publication of the Essay that Parkinson's name became permanently associated with the condition, formerly referred to as *paralysis agitans*, following acceptance by preeminent neuroscientists of the time such as Charcot and Gowers (Charcot 1861; Gowers 1893).

Following the devastating epidemics of influenza in 1918-1919, a population of patients exhibiting peculiar parkinsonian symptoms began to present at neurological clinics throughout Europe, Australia and the Americas. In addition to parkinsonian motor symptoms, cranial nerve palsies, oculogyric crisis and severe somnolence were common components of this disease. It was initially described as a form of “chronic encephalitis,” and was only later to be described as postencephalitic parkinsonism, or *encephalitis lethargica*. One of the eminent neurologists of the period, Dr. Constantin von Economo, presented

his description of the condition to the Vienna Psychiatric Society in April of 1917. As early as 1916, he described the typical syndromes in a monograph: “It seems strange when sleep appears as a symptom of an illness. ‘Sleeping sickness’ were the phenomenon of people fallingasleep while eating or working was first described in two cases in our clinic in 1916. Usually headache, nausea and fever were followed, often the next day, by sleepeing, frequently in a most uncomfortable position. One can wake them, but in severe cases, coma can rapidly lead to death. Malfunction of eye muscles, especially oculomotor dysfunction, and ptosis, was common.” Von Economo was quick to obtain autopsy data from severe cases, noting inflammation in the tegmental gray matter. He classified the disorder into three primary forms: somnolent-opthalmoplegic, hyperkinetic, and amyostatic-akinetic, which bore the hallmarks of parkinsonism. This amyostatic form was “characterized by a rigidity, without a real palsy and without symptoms arising from the pyramidal tract. This form of encephalitis lethargica is particularly common in the chronic cases, dominating the clinical picture as Parkinsonism. I reserve the name ‘Parkinsonism,’ though symptomatically identical with the amyostatic-akinetic form, rather for the chronic cases. To look at these patients one would suppose them to be in a state of profound secondary dementia. Emotions are scarcely noticeable in the face, but they are mentally intact.” (von Economo, 1931).

Some efforts have been made to link instances of encephalitis lethargica, both historical and contemporary, with infection by a viral agent, as it was widely assumed that the influenza epidemic of 1918 was related to most of the cases of

encephalitis presenting in neurological clinics at the time. As of this writing, no traces of influenza infection have been discovered in pathological specimens from patients who died of encephalitis lethargica in the 1920's through RT-PCR based testing, and its causative agent remains a mystery. The large number of patients that were brought to clinical attention with these symptoms did serve to push "Parkinsonism" into the consciousness of physicians of the time, and helped to solidify its standing as a clinicopathologic entity.

PD Therapy

A considerable period of time elapsed following the publication of Parkinson's essay before any effective therapy for PD was ascertained. Nevertheless, any rational discussion of PD as a modern clinical entity must be centered on the remarkable evolution of PD therapies over the last century. An examination of neurology textbooks of the late nineteenth century provides a pharmacologic laundry list of preparations administered to PD patients: opium, ergot, strychnine, datura, cannabis, hyoscyamine and belladonna among many others(Peterson 1890). It was around this time that anecdotal evidence began to accumulate suggesting some efficacy of belladonna extract in providing a minimal, yet consistent level of relief of symptoms. Following the large increase

in clinic populations during the early twentieth century of patients suffering from postencephalitic parkinsonism, a large variety of compounds were again administered on an experimental basis, with the best results again obtained through the use of belladonna extract. Patients with postencephalitic parkinsonism seemed to enjoy a greater therapeutic response to belladonna than did those with classical PD, though the doses used were very high compared with past treatment regimens. The Bulgarian physician Raeff produced a wine extract of belladonna root in the early 1920's, which attained widespread popularity in the treatment of postencephalitic parkinsonism as the "Bulgarian treatment." Variations of this extract were marketed in Europe and America into the 1940's. As with earlier extracts of this type, central and peripheral anticholinergic side effects were quite common, particularly at high dosages.

The usage of natural belladonna extract as a treatment of choice for PD was eroded by the production in the 1950's of a series of manmade anticholinergic compounds derived from atropine. The first of these compounds to be employed in PD therapy was caramiphen in 1946; it is no longer produced. One of the most potent to be synthesized, benztropine, is still in limited clinical use for PD. The compound that gained the greatest clinical usage in the 1950's was the piperidine compound trihexyphenidyl. It is still in use as an adjuvant to L-dopa therapy. Some limited therapeutic effects were discovered for the common antihistamine diphenhydramine (Benadryl) and its analogous compounds, but these were never widely exploited for PD therapy. Early compounds based on phenothiazine, later the substrate for the development of

the revolutionary tricyclic drugs such as chlorpromazine, also saw usage in PD. At least two of these, diethazine and ethopropazine, were marketed specifically as antiparkinsonian agents. Ethopropazine is still occasionally prescribed as an adjuvant to L-dopa therapy.

One of the major extrapyramidal side effects of the use of high dosages of chlorpromazine and the related tricyclic compounds was a form of parkinsonism. This observation fueled enthusiasm in the PD research community that elucidation of the biochemistry of the disease was a possibility (Merritt 1956). Following the initial investigations of the functions of acetylcholine in the CNS, Feldberg proposed in 1945 that the therapeutic value of scopolamine and atropine in PD was due to "central atropine-acetylcholine antagonism." Studies showed that the amount of antiacetylcholine activity of these compounds as measured in rodent systems correlated with their level of antiparkinsonian activity in humans. This notion was lent additional experimental support by the finding that physostigmine administration worsened parkinsonian symptoms, and could counteract the beneficial effects of benztropine in a dose-dependent manner. These experiments were key to a primary hypothesis of the biochemical nature of PD, namely that loss of activity of inhibitory dopaminergic inputs to the striatum resulted in a relative disinhibition of cholinergic neurotransmission.

The use of amantadine in PD therapy was initiated after a report (Schwab et al, 1969) detailing the improvement of symptoms in a patient initially given the compound as a prophylaxis against influenza. Amantadine was approved by the FDA for PD therapy in 1971. It has been shown to exert an indirect

anticholinergic effect by reducing conductance of the neuronal acetylcholine receptor (Moresco et al, 2002).

The late 1950's saw a series of revolutionary developments in the pharmacological treatment of PD. Dopamine was first discovered in mammalian brain homogenates by Montagu among others in 1957 (Montagu 1957). Carlsson, who confirmed the presence of dopamine in rodent brain homogenates in 1958, also demonstrated that administration of levodopa, a naturally occurring compound found in very high abundance in fava beans, was able to reverse the parkinsonian catalepsy induced by reserpine administration (Carlsson et al, 1958). In 1959 Bertler and Rosengren detailed the striatum-specific localization of dopamine in dogs, and Sano's group confirmed a similar localization in humans (Bertler and Rosengren, 1959; Sano et al, 1959). Ehringer and Hornykiewicz in 1960 were the first to describe a profound depletion in striatal dopamine in PD patients (Ehringer and Hornykiewicz, 1960). The stage was now set for the proposals set forth by Hornykiewicz and Carlsson to treat PD patients with levodopa, the blood-brain barrier permeant precursor to dopamine. In 1961, several patients at the Municipal Home for the Aged in Vienna were injected with 50 to 150 mg of levodopa, and "abolition or substantial relief of akinesia" was the result (Birkmayer and Hornykiewicz 1961). This initial success was followed by five years of administration of different D and L-dopa formulations, at dosages generally in the range of 50-300 mg , to PD patients worldwide. The heterogeneity of responses to these regimens considerably decreased the enthusiasm of most neurologists for dopa therapy, as evidenced by a 1965

review in which Duvoisin wrote, “Despite enthusiastic claims of therapeutic benefit, no evidence has been presented that the DOPA effect is in any way specific or that it differs from the effect of other sympathomimetic amines” (Duvoisin 1965).

This pessimistic attitude was to change in relatively short order, with the publication detailing the results of administration of high oral doses (3-16 g daily) of racemic D,L-dopa to 16 patients (Cotzias et al, 1967). Eight patients experienced “complete, sustained disappearance or marked amelioration of their individual manifestations of Parkinsonism.” Among the other patients, two exhibited improvement but not abolishment of motor symptoms. A gradual increase in the daily dose, 0.2 mg/day as opposed to a larger increment of 0.5 mg/day, substantially reduced the nausea, vomiting and disorientation previously observed with high dose dopa administration. Over the next several years, multiple studies confirmed these results, with most reports describing 75-80% of patients as having significant improvement of PD symptoms. Despite the side effects that were found to occur, such as hyperkinetic dyskinesias, these new regimens represented a quantum leap in the management of PD. Levodopa remains the most widely used pharmacologic treatment of PD to the present day.

The other major class of pharmaceutical compounds used in treating PD is dopamine agonists. These compounds act through association with D1 or D2 dopamine receptors which are linked to activation or inhibition of adenylate cyclase, respectively, and opening of potassium channels in the case of D2 receptors. The first generation of dopamine agonists were derivatives of ergot

(bromocriptine, lisuride and pergolide) and have been generally replaced by two synthetic agonists pramipexole and ropinirole.

With regards to initial therapy for a newly diagnosed case of PD, the primary choice to be made concerns use of a dopamine agonist versus L-dopa based therapy. Owing to the tendency of the L-dopa side effect profile to increase and therapeutic efficacy at a given dose to decrease with ongoing administration, with noticeable deterioration of response in two to five years, many practictioners seek to delay beginning this therapy while symptomatic relief can be obtained through the use of dopamine agonists, such as pramipexole and ropinirole. Several clinical trials randomly assigning patients to L-dopa or dopamine agonist therapy have demonstrated that the agonists produce significantly less incidence of dyskinesias, while L-dopa treated patients tend to have better overall motor function. This likely represents a therapeutic tradeoff, and the likely trend in the near-term for initial treatment of PD is likely to favor the dopamine agonists, especially for early-stage patients without significant motor symptoms.

There have been surgical attempts to address PD symptoms of rigidity and tremor through the years, ranging from wholesale extirpation of the motor cortex in the early twentieth century (producing paralysis, but definitively abolishing tremor), evolving into thalamotomies and pallidotomies, designed to interrupt the prokinetic cortex-striatum-pallidum-thalamus-cortex neuronal loop and generally are highly effective in abolishing severe medication-resistant tremor but are overall less effective at treating other PD symptoms. More

recently, the use of deep brain stimulation techniques, in which a high-frequency electrical device is surgically implanted in either the globus pallidus or subthalamic nucleus, have produced favorable short term results (Olanow, 2002).

Another recent development in the PD therapeutic armamentarium has been the implanation of fetal neurons into the nigra, with the hope that environmental cues would induce the fetal cells to differentiate and act as replacements for dopamine neurons lost. In a recent report by Freed et al, forty patients were randomly assigned to fetal cell implantation or sham procedure groups, with the sham group being offered the fetal cells at the conclusion of the twelve month study period (Freed et al, 2001). The results of the trial were disappointing, with no significant differences reported in patient-assessed severity of symptoms between the experimental and control groups. Despite this lack of symptomatic improvement, they observed a 40% increase in fluorodopa uptake in the nigra of the fetal cell group as compared to a slight decrease in uptake in the control group, as assessed by positron emission tomography. Additionally, severe dystonias and dyskinesias were associated with a significant number of the transplanted patients (5/33). The very recently reported results of the other major US trial of fetal brain transplantation showed similarly negative outcomes in terms of improving Parkinson's symptoms (Olanow, et al, 2003). These results have made fetal cell implantation a less attractive therapeutic option for now, especially in light of the ethical concerns being raised regarding the use of cells of fetal origins in all venues of biomedical research.

PD Pathology

Pathologically, PD is characterized by loss of the pigmented dopaminergic neurons of the substantia nigra (SN), and formation of Lewy bodies, eosinophilic cytoplasmic inclusions comprised of misfolded aggregates of alpha synuclein, neurofilaments, tubulin and ubiquitin. Lewy bodies are usually most prominent in the substantia nigra pars compacta and locus coeruleus of PD patients. The loss of neurons in the SN follows a characteristic pattern, with most loss occurring laterally in the ventral part of the SN pars compacta (Hirsch 1988). The loss of dopaminergic neurons results in a depletion of dopamine within the basal ganglia (Ehringer and Hornykiewicz, 1960), and it is this depletion that is responsible for production of the motor symptoms of the disease (Lee et al, 1994). Neurodegeneration occurring in locations outside the SN, including the ventral tegmental area, locus coeruleus and the basal nucleus of Meynert are thought to contribute to the cognitive Parkinsonian symptoms, such as dementia, that occur in around 30 percent of PD cases (Agid et al, 1990; Greenfield and Bosanquet, 1953; Candy et al, 1983; Aarsland et al, 1996). PD symptoms do not manifest themselves clinically until loss of 60 to 80 percent of SN dopaminergic neurons has occurred, generating widespread acceptance for a long presymptomatic period during which neuronal death is occurring but gross motor symptoms are not manifested. During this preclinical phase of the disease process, compensatory mechanisms such as increased expression/sensitivity of

dopamine receptors on remaining neurons are thought to prevent gross motor deficits (Agid et al, 1990). It is this preclinical phase and the compensatory capacity of the basal ganglia that it implies that causes some investigators to believe that a PD "therapeutic window" exists, during which it would be possible to slow or stop the neuronal loss and thus prevent most or all of the debilitation associated with the disease.

A major issue that frequently complicates discussions of PD nomenclature is the teleological distinction that must be made between PD and parkinsonism, which refers to the individual cardinal motor symptoms of the disease. Classic idiopathic PD, referring to the cardinal symptoms in the presence of autopsy-verified pathology of the substantia nigra with the presence of Lewy bodies, is distinct from the symptoms of parkinsonism, which can be individually produced by known insults to specific areas of the CNS. Parkinsonism can result from exposure to infectious agents (as in the epidemics of encephalitis lethargica mentioned above), environmental toxins (MPTP, manganese, CO, methanol, cyanide compounds), medications (neuroleptics, reserpine, lithium), organic disturbances of the CNS (stroke, trauma, increased intracranial pressure, tumor, subdural hematoma), genetic syndromes (Hallervorden—Spatz disease, Pick's disease, Wilson's disease, Huntington's disease) and even metabolic disturbances (hypoparathyroidism, hepatic coma) (Di Monte et al, 2002; Poewe and Wenning, 2002).

Genetic and other risk factors

Although the characteristic neuropathology of PD has been well known for decades, intensive research efforts have yet to elucidate the cause of death of SN dopaminergic neurons in PD. It has long been suspected that genetic predispositions to the disease may play a role, and investigations into nuclear and mitochondrial DNA mutations are ongoing. The most comprehensive survey of genetic contribution to idiopathic PD to date was carried out by Tanner et al, and comprised an analysis of members of the National Academy of Sciences/National Research Council World War II Veteran Twins Registry (Tanner et al, 1999). Out of 19,482 white male twins screened, 268 suspected cases of PD were identified. A total of 161 twin pairs in which 1 or 2 twins had PD were found. The overall concordance rate was similar for monozygotic versus dizygotic twins (15.5% and 11.1% respectively) in cases of PD diagnosed after 50 years of age. In cases diagnosed prior to 50, all examined monozygotic twin pairs (4 of them) were concordant for PD, versus 2 of 12 dizygotic twin pairs. These results were interpreted by the authors to suggest that genetics do not play a major role in PD with a typical age of onset, but may play a significant role in PD cases with a younger age of onset. Conversely, a study by Sveinbjörnsdottir and colleagues using an extensive Icelandic genealogic database found a possible effect for typical age of onset PD (Sveinbjörnsdottir et al, 1999). Briefly, those persons determined to have PD had a higher degree of interrelatedness than those unaffected. One must consider, however, that the

Icelandic population has a fairly low degree of genetic diversity overall, which may bias the results somewhat.

Mutations in genes such as Parkin, an E3 ubiqutin ligase, have been characterized and found to play a significant role in juvenile-onset forms of the disease, but such rare genotypes do not account for the vast majority of PD, the non-familial or sporadic forms of the disease. Lucking and colleagues screened 73 families with PD with atypical age of onset for mutations in the parkin gene. Parkin mutations were found in 49% of the families screened. The individual mutations varied widely with 19 homozygous and heterozygous exon rearrangements being found, as well as 16 different point mutations (Lucking et al, 2000). This study suggests that parkin mutations may play a prominent role in PD with early age of onset. In 1997, a study demonstrated linkage with the α-synuclein gene, located on chromosome 4q21-23, in an Italian-Greek family with early onset PD (Polymeropoulos et al, 1997). Despite this, α-synuclein mutations have not been found to be common in early onset PD, and have not been documented in any case of idiopathic PD. It is possible that certain alleles of these genes in combination with others may contribute to idiopathic PD by enhancing vulnerability to environmental or toxic factors.

There exists a considerable body of literature regarding the effect that lifestyle and environment may play in the development of PD. Historically, increased risks of developing PD have been associated with pesticide exposure, rural versus urban residence, consumption of well water and not smoking tobacco products. One recent study in humans described the reduction in risk of

PD in over eight thousand Japanese-American males associated with increased caffeine consumption (Ross et al, 2000). The relative risk of developing PD for those that did not consume coffee was 2.2 times that of the coffee drinkers, with an increase of 5 times for nonconsumers verus those that consumed seven or more cups of coffee (or equivalent amounts of caffeine) per day.

Other hypotheses proposed for the etiology of PD include an acceleration of the normal processes of aging of CNS neurons, exposure to endogenous or exogenous toxins (the environmental hypothesis), and alterations of protein handling leading to cytotoxic misaggregation. An intriguing alternative school of thought holds that PD is rather multifactorial, with genetic predispositions to neuronal death combining with circumstances of environment or lifestyle to produce the neuropathology and symptoms of the disease. As the extreme heterogeneity of the disease has been extensively illustrated, and studies attempting to evaluate concordance of PD in monozygotic twins have not proven conclusive, we may begin to articulate the idea of PD as a range within the spectrum of neurodegenerative disease, a pathology that represents a common neuronal response to many different circumstances. If we wish to move forward in our investigations under the assumption that there is likely to be no single cause for the majority of cases of PD, we must modify our experimental strategies accordingly. Just as the therapy of AIDS has been revolutionized by the usage of “cocktails” of multiple drugs to prevent HIV replication, greater success than has heretofore been attained in neuroprotective trials for PD drugs may be obtained by using them in combination rather than singularly. In the

same spirit, molecular investigations of the causes of the selective cell death in the substantia nigra should incorporate the testing of the contributions multiple gene products in parallel rather than singular genes or proteins in isolation. While this mode of thinking may at face value seem to flout the traditional imperative of reductionism in modern biology, this is not the case; the objective of any such multifactorial investigation, regardless of the number of molecular entities involved or the volume of data generated, must be the elucidation of the common pathways and cascades that can synthesize such diverse biological inputs into the final output of selective dopaminergic neuronal loss. A starting point for our investigations has been a common paradigm of many of the leading hypotheses of the etiology of PD: oxidative stress.

Oxidative Stress in PD

Oxidative stress is defined as an overabundance of reactive oxygen species (ROS) relative to the cell's antioxidant capabilities, leading to ROS attack on proteins, lipids and nucleic acids. Multiple sources of intracellular ROS have been described, the most important of which by far are the mitochondria. Mitochondria are well known to every student of biology as the primary source of ATP in eukaryotic cells through the action of the electron transport chain (ETC), and account for 85 to 90 percent of the oxygen used by a typical cell under physiological conditions (Chance et al, 1979). Less well articulated in many

courses of study is the concomitant production of ROS as a byproduct of this oxygen use. While mitochondria have been shown to have antioxidant mechanisms in place to deal with some of their own ROS production (and that the presence of these mechanisms are essential to cell survival; Melov et al, 1998), it has also been demonstrated that a typical mitochondrion produces ROS at a rate that exceeds the ameliorative capacity of these enzymes. This results in the incomplete usage of 1 to 3 percent of oxygen consumed by the cell (Boveris and Chance, 1973).

These altered stochiometries of electron flow between the protein complexes of the mitochondrial electron transport chain are thought to be responsible for generation of such compounds as hydrogen peroxide, superoxide radicals and hydroxyl radicals. Although the former two species are not extremely deleterious to the cell, with specialized enzyme defenses designed to catalyze their degradation, they are capable of reacting with other elements commonly found within the cell to form significantly more dangerous compounds.

A body of experimental evidence documenting the increased appearance of markers of oxidative damage in PD postmortem brain samples as compared to controls has accumulated over the last 10-15 years. Most of these studies have focused on immunohistochemical methods of detection of ROS-mediated damage to cellular lipids, proteins and/or nucleic acids. Alam et al have described increased levels of protein carbonyl groups (a marker for ROS attack on histidine, arginine, lysine and proline residues) in SN, caudate and putamen of PD postmortem brains compared to controls (Alam et al, 1997). Good et al have

reported the presence of nitrotyrosine residues, produced by peroxynitrite radical attacks upon tyrosine amino acid residues, within the cores of Lewy bodies in PD brain samples (Good et al, 1998). This indicates that peroxynitrite acts within the vulnerable/dying neruons selectively affected by PD. Increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a product of nucleic acid oxidation, have been found in SN of PD patients (Alam et al, 1997; Zhang et al, 1999). Additionally, Kikuchi et al found increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8-OHG), products of reactive oxidization of nucleic acids in serum and cerebrospinal fluid of PD patients compared to controls (Kikuchi et al, 2002), indicating that this finding was not an exclusively postmortem phenomenon. This finding was recently independently confirmed (Abe et al, 2003). The levels of polyunsaturated fatty acids, the substrate for ROS peroxidation of cellular lipids, are decreased in several brain regions of PD postmortem brains relative to controls; the same study confirmed that levels of malondialdehyde, an intermediate compound in lipid peroxidation, are increased in the same PD postmortem brains (Dexter et al, 1989). Taken together, this likely indicates that lipid peroxidation by ROS proceeds at an elevated rate in PD. Additionally, Yoritaka et al have documented the greatly increased presence of 4-hydroxy-nonenal (HNE) protein adducts in PD nigral neurons compared to controls. HNE is an unsaturated aldehyde species that is one of the major products of membrane lipid peroxidation and is reported to inhibit nucleic acid and protein synthesis and interfere with heat shock protein and other enzyme induction (Yoritaka et al, 1996).

Ubisemiquinone produced by the normal processes of the ETC is the primary electron donor responsible for the generation of superoxide radicals in mitochondria (Turrens et al, 1985). The superoxide radical attacks enzymes that contain iron sulfur centers, such as aconitase, succinate dehydrogenase and (in the mitochondria) NADH-ubiquinone oxidoreductase (Pryor, 1986; Gardner et al, 1995). Due to the threat posed to operation of the ETC by its presence, mitochondrial superoxide is rapidly converted to hydrogen peroxide by the action of mitochondrial manganese superoxide dismutase (SOD2). Two other superoxide dismutase enzymes are present in mammalian cells, SOD1, a copper-zince superoxide dismutase present in the cytosol, and SOD3, another copper-zinc dismutase that is expressed extracellularly (Turrens et al, 1985). In SOD2 knockout mice, mitochondrial superoxide radical is not converted into hydrogen peroxide and causes extensive damage to complex I of the ETC, NADH-ubiquinone oxidoreductase, succinate dehydrogenase and aconitase, all of which have iron-sulfur moieties. This ETC damage leads to a reduction in ATP production and increased lactic acid production. The mice develop lactic acidosis, cardiomyopathy and degeneration of the basal ganglia, closely mimicking symptoms seen in humans with inherited ETC mutations. Mice that lack the copper-zinc superoxide dismutases are not affected at birth, but develop axonal neurodegeneration later in life (Li et al, 1995; Lebovitz et al, 1996).

While hydrogen peroxide, chemically, is not a true oxygen radical (it lacks unpaired electrons), it is significant in the ROS picture due to its ability to be enzymatically transformed into many different derivatives, and its ability to diffuse

across several cell radii. Hydrogen peroxide in combination with ferrous iron or other metals can create hydroxyl radicals by the Fenton reaction (Raha et al, 2000). Hydroxyl radicals are extremely reactive, with a very short half life. They cause peroxidation of lipids, proteins and nucleic acids. The primary means of limiting hydroxyl radical toxicity is to limit the production of hydrogen peroxide and the availability of free transition metals (i.e., substrates for the Fenton reaction). As the production of hydroxyl radicals is highly hazardous to the cell, most hydrogen peroxide generated in the mitochondria is processed by glutathione peroxidase (GPX) into water (Beyer et al, 1991).

The other major ROS produced in the mitochondria is peroxynitrite. It is formed by the reaction of superoxide radical with nitric oxide (NO). Recent evidence demonstrates that mitochondria possess their own nitric oxide synthase (mtNOS), a sub-isoform of neuronal nitric oxide synthase. At physiological levels, NO inhibits the opening of the mitochondrial permeability transition pore (MTP), while higher levels promote its opening (Brookes et al, 2000). NO is also capable of influencing the ETC by reversible inhibition of cytochrome c oxidase. It is a highly diffusible compound that freely crosses lipid membranes, therefore if a cell produces abnormally high levels of NO it is theoretically capable of inhibiting respiration within neighboring cells. This property may explain varying degrees of damage to neuronal ETCs that develops when astrocytes in combined neuron-astrocyte cultures produce excess NO (Bolanos et al, 1995). In the astrocytes, glutathione (GSH) is preferentially depleted (Ju et al, 2000).

Peroxynitrite radical, while not as damaging as hydroxy radical, is nevertheless highly reactive and dangerous. It modifies proteins by nitrosylation of tyrosine residues and by oxidizing tryptophan and cysteine residues (Ischiropoulos and al-Mehdi, 1995). Within mitochondria, it is particularly damaging to complexes I, II, IV and V of the ETC, as well as aconitase, SOD2 and creatine kinase. It can also react with mitochondrial lipid membranes and mitochondrial DNA, which has no protective histone scaffolding (as nuclear DNA does) to shield it from such attack (Brown and Borutaite, 1999). Damage to these molecules can induce mitochondrial swelling, calcium release and opening of the MTP. Mitochondrial calcium uptake has been shown to induce mtNOS, leading to a concomitant increase in peroxynitrite radical and release of calcium; this is hypothesized to consitute a feedback loop preventing excessive accumulation of mitochondrial calcium. These studies demonstrate that excessive production of ROS by mitochondria can cause extensive damage to the brain among other organs.

It is currently a matter of active debate as to whether manifestations of oxidative stress represent a causative factor for dopaminergic cell death or simply a byproduct of such neuronal loss. This distinction is critical; the merits of continued study of this question are clear, as even if induction of ROS is a downstream event from initial dopaminergic cell losses, it may promote a reactive environment that weakens the remaining neurons and contribute to their eventual demise.

It has been suggested that SN dopaminergic neurons exhibit a set of predisposing factors that make them particularly vulnerable to increases in ROS. While the normal catabolism of dopamine itself through the action of monoamine oxidase leads to hydrogen peroxide formation, nonenzymatic breakdown of dopamine within the neuron produces neuromelanin pigment, which can in turn promote the production of hydroxyl radicals when exposed to free iron (Fahn and Cohen, 1992; Jellinger et al, 1992). Hirsch, et al. showed that indeed, the SN neurons most susceptible to neurodegeneration were those with relatively higher levels of neuromelanin (Hirsch et al, 1988). Some researchers have inferred that the subcellular localization of the neuromelanin within the phagolysosomic membrane compartments represent an attempt by the cell to protect itself from its own neurotransmitter metabolism, with the lipofuscin serving as a “sponge” for excess oxygen radicals or other endogenous toxins (Brunk and Terman, 2002).

Another primary factor underlying neuronal viability to ROS is the relative scarcity of antioxidant enzymes (AOE) in the brain relative to other tissues. Potential relevance to PD was described when a study reported reduction in the reduced form of glutathione in the SN of PD postmortem brain samples (Perry et al, 1982), later independently confirmed (Sofic et al, 1992; Pearce et al, 1997). The reduction of glutathione levels is apparently most prominent in glial cells, perhaps reflective of transport of the glutathione from glia to the more highly “threatened” dopaminergic neurons. This notion is supported by the finding of an increase in glial gamma-glutamyl transpeptidase, an enzyme involved in the production of active glutathione. Additonally, a global decrease in glutathione

levels as opposed to a selective depletion of the reduced (GSH) form, would be expected to manifest itself in an unchanged reduced:nonreduced glutathione ratio; in SN of PD patients, GSH is reduced without significant changes in the nonreduced form, implying the presence of increased oxidative stress (Kish et al, 1985). These drops in GSH are often accompanied by reductions in catalase and GSH peroxidase expression (Ambani et al, 1975; Kish et al, 1985), which further predispose the cells to oxidative damage.

Another potential source for ROS in the SN comes from the activation of microglial cells. In response to increased levels of ROS in the extracellular milieu, these cells are induced to produce cytokines and nitric oxide. Expression of the inducible nitric oxide synthase (iNOS) has been found to be upregulated in PD brain generally (Hunot et al, 1996). This in turn leads to impairment of ETC and enhanced generation of peroxynitrite species by the methods previously described.

Increased levels of iron have been reported in SN pars compacta of PD patients and in neuromelanin-containing vesicles of dopamine neurons (Dexter et al, 1989; Hirsch et al, 1991). Although the cause of the increased levels are unclear, a decrease in cellular ferritin and/or an increase in lactoferrin receptor expression may be partly to blame (Riederer et al, 1989; Dexter et al, 1990; Leveugle et al, 1996). This increase in iron levels could theoretically contribute to increased ROS in these neurons, via increased availability of substrates for combination with hydrogen peroxide in Fenton reactions.

Parkinsonian neurotoxins

Perhaps no event revolutionized the oxidative stress hypothesis of Parkinson's disease more than the discovery of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). MPTP is a widely used chemical feedstock that was accidentally produced as a byproduct in the synthesis of an analogue of meperidine (Demerol). The MPTP-contaminated drug was sold illicitly in northern California in the early 1980's. The drug abusers that injected this compound, generally in their early 20's, began to exhibit acute and chronic symptoms typical of long standing PD in an aged patient. The patients that came to medical attention generally reported burning at the injection site, followed by a metallic taste in the mouth, jerky motions of the extremities and a variety of sensory abnormalities including "dreamy or spacey feelings," hallucinations and loss of vision. These symptoms generally disappeared within one to two hours following injection, depending on the dose administered. Those that used the compound repeatedly developed a series of chronic symptoms, including stiffness of the muscles, slowness of movements (bradykinesia) and resting tremor. Physical examination of the subjects revealed "virtually all of the typical features of PD" (Langston et al, 1983). Although the subjects did not suffer from the dementia that often accompanies advanced PD, Langston et al did note minor abnormalities of mental functioning consistent with nondemented patients with PD of long standing. The similarities of the group to idiopathic PD patients

extended to their response to therapy: the motor symptoms they experienced could be alleviated with L-dopa therapy, with the accompanying “on-off” effects and dyskinesias seen in PD patients nearing the end of the period of effective L-dopa therapy. As these patients were relatively young and otherwise healthy, the pathologic lesions responsible for their symptoms were not confirmed by autopsy. Tragically, this data did become available when a student at the University of Maryland began to synthesize and self-administer an opiate analog. He also developed parkinsonian symptoms, following a failed synthesis (Davis et al, 1979). This troubled person eventually committed suicide, and upon autopsy substantial loss of cells in the substantia nigra was observed, virtually identical to the cellular pathology observed in advanced PD.

One of the most fundamental differences between MPTP-induced Parkinsonism and idiopathic PD is that the former is an acute syndrome, manifesting within hours-days-weeks, whereas the latter is a chronic process that requires decades to produce the characteristic clinical syndrome. Despite this major difference, MPTP remains one of the best-studied animal models of PD.

The presence of the lipophilic phenyl group aids MPTP in crossing the blood-brain barrier. Once inside the CNS, MPTP is taken up by glial cells, where formation of the toxic metabolite methylpyridinium ion (MPP+) is catalyzed by the enzyme monoamine oxidase B (Heikkila et al, 1984). MPP+ is then selectively accumulated by SN dopaminergic neurons by virtue of its affinity for the dopamine transporter (Javitch et al, 1985). Once inside the neuron, MPP+ is accumulated by the mitochondria, where it potently inhibits complex I of the

electron transport chain (Hasegawa et al, 1990). It is hypothesized that alteration of the stochiometry of electron transport through complex I interference is responsible for an increase in ROS formation by the neuron in question, leading to oxidative intracellular damage eventually culminating in the observed cell death. Przedborkski and colleagues reported that transgenic mice engineered to overexpress the superoxide dismutase SOD1 were more resistant to MPTP intoxication than wild-type mice, bolstering the notion of MPTP's induction of ROS (Przedborski et al, 1992). As noted, SOD1 acts primarily to catalyze the dismutation of the superoxide anion into hydrogen peroxide. As the superoxide anion is not highly toxic *in vivo*, attention must be paid to a highly toxic product of superoxide's reaction with nitric oxide: peroxynitrite. This idea has been lent support primarily by the group of Flint Beal, who have noted severally that specific inhibitors of the neuronal nitric oxide synthase (nNOS) are protective against MPTP intoxication and nigral cell death in rodents, and that knockout mice lacking nNOS are also protected against MPTP's effects relative to wild-type mice (Grunewald and Beal, 1999; Matthews et al, 1997; Przedborski et al, 1996; Schulz et al, 1995). A reactive gliosis of the substantia nigra and striatum is a prominent feature of MPTP intoxication in mice (Dehmer et al, 2000), as was noted by Langston in humans(Langston et al, 1999). Apart from this proliferation, the glial cells in the vicinity begin to express inducible nitric oxid synthase (iNOS). Similar to the situation with nNOS, neurons die at a much reduced rate in the substantia nigra of iNOS knockout mice, but dopaminergic fibers are observed to atrophy and dopamine stores are depleted (Dehmer et al,

2000; Itzhak et al, 1999; Liberatore et al, 1999). NOS inhibitors have been observed to block the neurotoxic effects of MPTP in nonhuman primate models (Hantraye et al, 1996). Finally, cellular markers of the deleterious effects of NO-based reactions, such as nitrosylation of proteins, as well as iNOS upregulation, have been observed in the substantia nigra of PD patients (Good et al, 1998). When taken together, this evidence argues strongly in favor of the involvement of NO in the neurotoxic effects of MPTP and its derivatives, and for this reason we decided to study the effects of nitric oxide modulation in our cellular model of MPP+ intoxication with studies involving application of the NO-scavenging agent PTIO.

Cell Death in PD

A controversial area of PD research deals with the manner in which neurons of the substantia nigra die. The paradigm of apoptosis, or programmed cell death, has dominated most of the research into this area of PD. Mochizuki reported TUNEL staining (indicative of DNA fragmentation) of nuclei in PD patients in 1996(Mochizuki et al, 1996). Ultrastructural analyses by Anglade et al reported typical apoptotic membrane blebbing and nuclear condensation in substantia nigra, with portions of pigment-containing neurons endocytosed by surrounding glial cells (Anglade et al, 1997). The most widely studied forms of apoptotic cell death involve the activation of a complex intracellular signaling cascade culminating in the activation of caspases, or cysteine-aspartate-serine

proteases. Activation of caspase 3, one of the principal downstream effectors of the process, has been reported by Oo et al in isolated dopamine neurons of the substantia nigra, and by Hartmann et al in PD dopaminergic neurons(Oo et al, 2002; Hartmann et al, 2000). The tumor necrosis factor cell death cascade culminates in the activation of caspase 8, which was also reported by Hartmann to occur to a greater extent in dopaminergic neurons from PD patients than in those of control subjects.

Evidence from several cell culture models, including SH-SY5Y neuroblastomas, indicates that dopamine itself is capable of inducing apoptosis (Ziv et al, 1994; Massenaro et al, 1996; Gabby et al, 1996; Junn and Mouradian, 2001). This effect is counteracted by overexpression of the antiapoptotic protein Bcl-2(Offen et al, 1997; Ziv et al, 1997). As the breakdown of dopamine has been noted to produce excess ROS burdens in the cell, it is presumed that this is contributory to the apoptotic death seen in these models. Studies by Barzilai et al detail the involvement of collapsin, a protein involved in axonal guidance, and TCP-1, a heat shock protein family member, in dopamine-mediated apoptosis in sympathetic neurons (Barzilai et al, 2000). The use of antibodies against collapsin and of antisense DNA for the TCP isoform delta have attenuated apoptotic death in these neurons. Dopamine is also capable of apoptotic induction in SY5Y cells, by the criteria of caspase 3 and 9 activation and nuclear condensation (Junn and Mouradian 2001). P38 MAP kinase activation and mitochondrial cytochrome c release were found to mediate this process in SY5Ys. MPP+ treatment has been demonstrated by Hartmann and others to

cause apoptosis in dopaminergic PC12 cells and in primary cultures of midbrain neurons (Hartmann et al, 2000; Viswanath et al, 2001). Previous work in our laboratory has demonstrated MPP^+ induced apoptosis in SY5Y cells (Fall and Bennett, 1999). This study further demonstrated that this apoptosis was directly related to excess production of reactive oxygen species through an inhibition of complex I of the mitochondrial electron transport chain; Rho^0 SY5Y cells, which lack mitochondrial DNA and do not carry out oxidative phosphorylation, do not undergo apoptosis when exposed to MPP^+. Work by Cassarino et al carried out at the University of Virginia Center for the Study of Neurodegenerative Diseases (CSND) has further demonstrated that such MPP^+ treatment leads to activation (through phosphorylation) of JNK kinase, JNK and c-jun, which are in turn responsible for the activation of the caspases that carry out apoptosis (Cassarino et al, 2000). Concurrent work by Halvorsen (Halvorsen et al, 2002) detailed the activation of nuclear factor kappa-beta and protein kinase B (Akt) early in the time course of exposure of SY5Y cells to MPP^+. The signaling cascades controlled by these molecules are generally considered to be anti-apoptotic; these results indicated that the response to MPP^+ in SY5Y cells may involve factors outside the realm of electron transport inhibition, and served to fuel my interest in determining what other signaling cascades might be operative in this situation.

Previous data have demonstrated that expression of p53, crucial to the apoptotic process, is upregulated in the striatum and midbrain of PD patients (Alves da Costa et al, 2002). Previous work in the CSND (Halvorsen et al, 2002;

Dennis and Bennett, 2003) has described the concurrent activation of both pro-apoptotic and anti-apoptotic signaling pathways in cellular models of PD. Alternatively, work by Jellinger (Jellinger and Stadelmann, 2000) and Banati (Banati et al, 1998) failed to note any significant morphological signs of apoptosis in PD brain compared to control. Some of the heterogeneity of results obtained by these studies must be due to variations in the techniques and standards used to define apoptotic versus nonapoptotic cells; the preponderance of biochemically based studies measuring pro and anti-apoptotic protein and mRNA levels in cell and animal models and in PD brain samples seems to favor the notion of apoptotic cell death as occurring in PD. This has served to generate interest in determining whether the rate of dopaminergic cell death can be reduced through administration of anti-apoptotic compounds. It is uncertain whether inhibition of the apoptotic cascades in nigral cells would be sufficient to prevent their death. Encouraging data on the apotosis-preventive properties of the compound CGP3466B, thought to act via inhibition of expression of the pro-apoptotic protein Bcl-2, has been obtained by different groups (Andringa et al, 2000; Waldmeier et al, 2000).

Technical approaches

A major technological advance that has greatly increased our ability to rapidly examine many different gene expression responses simultaneously is the cDNA microarray. In its most basic form, the array consists of an ordered grouping of DNA that is adsorbed to a non-permeant surface material. When these fixed DNAs are exposed to homologous DNAs in solution, hybridization between the DNA species occurs. Generally, the DNAs that are washed over the array are labeled to allow detection by fluorescence, excited particle emission, etc. With this experimental paradigm, it is possible to examine the relative levels of particular DNA molecules within a sample in a highly parallel manner (limited by the number of genes in the fixed array). The first type of cDNA microarray to become commercially available involved 300 to 400 base pair cDNAs, selected for lack of secondary structure and uniqueness of primary sequence, covalently bonded to a nylon membrane. Following isolation of total RNA or mRNA from an experimental sample, a pool of cDNAs representative of the sample would be generated by a reverse transcription reaction using the mRNA as substrate. Generally, a radioactive nucleotide, usually ^{32}P or ^{33}P, would be incorporated into these so-called “first strand” cDNAs. These radioactive probe cDNAs would then be applied in solution to the nylon membrane bearing the target cDNAs, and hybridization at controlled temperature would occur. Following a series of washes, hybridization of the probe to target would be visualized by a

standardized method, most often storage phosphor imaging but sometimes x-ray film exposure. This method had the advantage of being highly replicable and straightforward to learn due to its commonalities with Southern and northern blot techniques. However, it suffers from a relatively small dynamic range and from the difficulties associated with the usage of radioactive materials. Its use has been largely supplanted by arrays spotted onto glass microscope slides, with fluorescent based detection methods replacing radioactivity.

One of the first methods for producing DNA arrays on glass slides was developed by Affymetrix Corporation, as a variation of the laser photolithography methods used to produce computer chips. In brief, synthetic linker molecules with photolabile attachment sites are attached covalently to the glass slide, then a mask is placed over the slide prior to exposure to laser light. This treatment makes the attachment sites so exposed available for reaction with bi-functional deoxynucleotides, thus beginning the process of building an oligonucleotide chain at a series of discrete spots on the array. The process of masking, laser exposure and addition of deoxynucleotides is repeated cyclically until an oligonucleotide of the desired length and sequence has been constructed. The most common physical standards for Affymetrix arrays are 1.28 x 1.28 cm, containing approximately 5×10^5 oligonucleotides, 25 base pairs in length. Approximately 15 oligonucleotides are constructed to study a given gene product, with oligonucleotide sequences overlapping one another within the target gene. Oligonucleotides are synthesized with 100% homology to the target sequence (perfect matches) or with single base pair substitutions (mismatches).

Following hybridization with fluorescently labeled probes, the signals observed in perfect match and mismatch oligonucleotides are compared; the signal from the mismatch oligos is intended to serve as a control for background noise and/or crosshybridization from other probes. This array technology has several significant advantages, including the ability to examine in excess of 30,000 genes on a single array, a (relatively) long period of use by the research community and a strong manufacturing base. However, they also have negative aspects; studies by Kuo and colleagues at the National Cancer Institute compared signals obtained from identical RNA samples derived from various cancer cell lines on cDNA type and Affymetrix type arrays, with very poor correlational results (Kuo et al, 2002). Additionally, the arrays are extremely costly, and this factor coupled with the difficulties in data analysis and comparison that are brought about by use of the perfect match/mismatch system has caused our laboratory to consider them as less than ideal for our intended work.

Our laboratory's interest in microarray technology has arisen from a continuum of work that has taken place over the last five to seven years, in which we have investigated the contributions of mitochondria, cell signaling pathways, and gene expression responses to apoptotic death in a cellular model of Parkinson's disease: SY5Y neuroblastoma cells exposed to the mitochondrial neurotoxin MPP+. The recent availability of high quality microarrays at a reasonable cost has made the investigation of the responses of thousands of genes to experimental perturbations in this system scientifically and economically feasible; we hope that the results that we obtain from such "gene discovery"

studies will provide us with the initial building blocks to construct an integrated theory for the dependence of cell death/survival on gene expression in such models, and in so doing, to begin to add quantitative methods to the validation of such models as they apply to Parkinson's disease *in vivo.*

Recent developments in gene array analysis of Parkinson's disease

Several groups have published results of cDNA microarray analyses of animal models of PD. Moussa Youdim's group at the Technion in Haifa, Israel administered MPTP to mice chronically and found significant up or downregulation of over 50 genes related to oxidative stress, inflammation, nitric oxide and glutamate metabolism, and neurotrophic factors in extracts from midbrain (Mandel et al, 2002). Most of these gene expression changes were prevented by administration of the D1-D2 dopamine receptor antagonist R-apomorphine, which also has ROS-scavenging properities. Administration of the enantiomer, L-apomorphine, which shares the ROS scavenging properties of the R-enantiomer but lacks its activity at dopamine receptors, had a virtually identical effect. This study demonstrated that MPP^+ administration can produce gene expression changes in a rodent model, and more importantly, that some portion of these gene expression changes are dependent on the status of ROS within the cell.

Brown and coworkers employed a new data analysis technique, that they call voxelation, in their study of whole brain gene expression in mice chronically administered methamphetamine. Briefly, ten coronal mouse brain slices were divided into four quadrants each, giving a total of 40 volume elements (voxels). 55 genes with highly correlated temporal expression patterns were differentially expressed between methamphetamine-treated and control mice, and the expression of each gene was determined for each voxel, providing spatial expression information (Brown et al, 2002). These genes had mainly neuronal morphology, intracellular signaling and apoptosis-related functions associated with them. This study was the first to suggest that a portion of the neuronal response to a parkinsonian neurotoxic insult might involve changes in morphology and cell-cell contact status.

Napolitano and colleagues recently published data concerning the gene expression response changes observed in the striatum of rats following nigral injection of the highly neurotoxic dopamine adduct 6-hydroxydopamine (6-OHDA) over a two month period (Napolitano et al, 2002). Significant alterations of expression of genes involved in transcriptional regulation and cell cycle regulation were observed. Specifically, the activity of the dopamine-PKA-Cdk5 cascade was severely downregulated, resulting in dephosphorylation of the DARPP-32 phosphoprotein that is thought to be a primary mediator of dopamine signaling. Additionally, genes involved in glutamate transmission of striatal neurons were significantly affected. This study, owing to its extended period of 6-OHDA administration, was the first to address what gene expression changes

might be involved in alterations of nigral neurotransmission in response to a parkinsonian neurotoxic insult.

Yoo and colleagues provoked oxidative stress in the dopaminergic neural cell line SN4741 by exposure to hydrogen peroxide or MPP+. 36 genes were identified that showed significant changes in response to the oxidative stimulus, including nuclear components of complex I of the mitochondrial electron transport chain, genes involved in membrane trafficking, oxidative stress markers and oxidoreductases (Yoo et al, 2003). Specifically, components B8 and B17 of mitochondrial complex I were downregulated in response to oxidative stress, while syntaxin 8 and heme-oxygenase 1 were upregulated. Heme oxygenase 1 has been previously demonstrated to be a major constituent of Lewy bodies, one of the pathognomic findings associated with idiopathic PD. In addition to performing their studies in a cell model of PD, this study was significant in that several of the genes showed changes in their temporal regulation over the period of following induction of oxidative stress (4-16 hours).

While a considerable corpus of experimental evidence for oxidative damage in the postmortem brains of PD patients exists, the relationship between this pathology, mitochondrial and other sources of oxidative stress and the neurodegeneration leading to the sporadic PD phenotype is highly complex and as yet incompletely understood. These relationships are nevertheless important and highly worthy of exhaustive investigation, as interventions against the processes that lead to PD may also have a place in the therapy of other common neurodegenerative conditions (Alzheimer's disease, amyotrophic lateral

sclerosis, etc.). Primary importance should be placed on the development of cell and animal models that more closely approximate sporadic PD than those currently available, and detailed investigation of genomic, transcriptomic, proteomic and intracellular signaling changes that are inherent to the pathology and symptomatology. Despite the recent completion of the Human Genome Project, fundamental gaps in our knowledge remain regarding the nature of the regulation of gene expression, sufficient to make a comprehensive evaluation of all expression events in a given cell or tissue impractical at this time.

I would like for us then to consider the experiments that I detail in the following chapters to be initial steps toward a deeper understanding of PD, moving beyond the clinicopathologic definitions of the disease to elucidation of the molecular mechanisms that are responsible for the death of dopaminergic neurons, the *sine qua non* of the disorder. These experiments could not and should not be construed as the end point of such endeavors, but hopefully as a guideline for future experiments that may examine the roles of many more gene products and their modulation in the context of parkinsonian insult, with our overarching objective as always being the discovery of new targets for therapeutic intervention in the disease process. The results that we have obtained represent a novel paradigm in the multiplex analysis of gene function in a widely used cellular model of Parkinson's disease.

RNA Interference

We began to seek out methods with which we could directly apply data gleaned from our array work to parametric evaluation of our cellular models of PD. One such logical avenue is the experimental perturbation of specific RNA signals previously observed in cells. Within the last three years, a significant body of publications has documented the newfound importance of RNA interference (RNAi), a form of post-transcriptional gene silencing. The effect was first observed by Jorgensen et al in their attempts to alter coloration in petunias by introducing a pigment producing gene under control of a constitutive promoter (Jorgensen et al, 1996). Rather than producing uniform deepening of pigmentation, a variety of striped patterns and even loss of endogenous pigment was observed. They named this phenomenon “cosuppression” due to the curious disruption of expression of both the introduced gene and the homologous endogenous gene. Since the original findings of Jorgensen were published, similar effects have been observed in many other species of plants as well as in fungi such as *Neurospora,* where Cogoni et al reported that the silencing effect could be transferred between nuclei in heterokaryotic strains (Cogoni et al, 1996). This particular result served to demonstrate that a trans-acting agent was involved. In some species of plants, the mechanism of silencing induced by transgenes appeared to be mediated by methylation of the homologous nuclear genes in question. In other species reduction of expression occurred at a post-

transcriptional stage, as the homologous transcript was produced but rapidly degraded.

The first evidence that dsRNA could lead to gene silencing came from work in the nematode *Caenorhabditis elegans*. Eight years ago, researchers Guo and Kemphues were attempting to use antisense RNA to shut down expression of the par-1 gene in order to assess its function (Guo and Kemphues, 1995). As expected, injection of the antisense RNA disrupted expression of par-1, but quizzically, injection of the sense-strand control did too. This result was a puzzle until three years later. It was then that Fire and colleagues first injected dsRNA, a mixture of both sense and antisense strands, into *C. elegans*. This injection resulted in much more efficient silencing than injection of either the sense or the antisense strands alone. Injection of just a few molecules of dsRNA per cell was sufficient to completely silence the homologous gene's expression (Fire et al, 1998). Furthermore, injection of dsRNA into the gut of the worm caused gene silencing not only throughout the worm, but also in its first generation offspring. The potency of RNAi inspired Fire and Timmons to try feeding nematodes bacteria that had been engineered to express dsRNA homologous to the *C. elegans* unc-22 gene (Timmons et al, 2001). Surprisingly, these worms developed an unc-22 null-like phenotype. Further work showed that soaking worms in dsRNA was also able to induce silencing. These strategies, whereby large numbers of nematodes are exposed to dsRNA, have enabled large-scale screens to select for RNAi-defective C. elegans mutants and have led to large numbers of gene knockout studies within this organism. Additionally, the effect

was found to be exquisitely sequence-specific; discrepancy of even a few base pairs between the dsRNA and the target mRNA virtually abolished the silencing. RNAi has been used experimentally in these non-mammalian systems to generate transient silencing of specific genes of interest, especially those which are not amenable to more traditional gene knockout methods (e.g., those that produce embryonic lethality and thus cannot be studied in the adult animal).

RNAi has also been observed in *Drosophila*. Although a strategy in which yeast were engineered to produce dsRNA and then fed to fruit flies failed to work, microinjecting *Drosophila* embryos with dsRNA does induce silencing. Silencing can also be induced by biolistic techniques in which dsRNA is "shot" into *Drosophila* embryos, or by engineering flies to carry DNA containing an inverted repeat of the gene to be silenced. Over the last few years, these RNAi strategies have been used as reverse genetics tools in *Drosophila* organisms, embryo lysates, and cells to characterize various loss-of-function phenotypes. Zamore's group found that dsRNA added to *Drosophila* embryo lysates was processed to 21-23 nucleotide species. They also found that the homologous endogenous mRNA was cleaved only in the region corresponding to the introduced dsRNA and that cleavage occurred at 21-23 nucleotide intervals (Haley et al, 2003). Current models of RNAi divide the process into broad "initiation" and "effector" stages. In the initiation step, input dsRNA is digested into 21-23 nucleotide small interfering RNAs (siRNAs), which have also been called "guide RNAs." Evidence indicates that siRNAs are produced when the enzyme Dicer, a member of the RNase III family of dsRNA-specific ribonucleases, processively cleaves dsRNA in

an ATP-dependent, processive manner. Successive cleavage events degrade the RNA to 19-21 bp duplexes (siRNAs), each with 2-nucleotide 3' overhangs.

In the effector step, the siRNA duplexes bind to a nuclease complex to form what is known as the RNA-induced silencing complex, or RISC. An ATP-depending unwinding of the siRNA duplex is required for activation of the RISC. The active RISC then targets the homologous transcript by base pairing interactions and cleaves the mRNA ~12 nucleotides from the 3' terminus of the siRNA. Although the mechanism of cleavage is at this date unclear, research indicates that each RISC contains a single siRNA and an RNase that appears to be distinct from Dicer. Because of the remarkable potency of RNAi in some organisms, an amplification step within the RNAi pathway has also been proposed. Amplification could occur by copying of the input dsRNAs, which would generate more siRNAs, or by replication of the siRNAs themselves. Alternatively or in addition, amplification could be effected by multiple turnover events of the RISC.

The presence of RNAi in mammalian cells was only recently established (Elbashir et al, 2001). Transfection of dsRNA in excess of 30 base pairs usually results in a non-specific “pan-suppression” of RNA transcripts. Two pathways contributing to this effect have been known for some time. In the first, the presence of the long dsRNAs activate the PKR protein kinase, which serves to phosphorylate and inactivate eIF2a, translation initiation factor (Manche et al, 1992). This has the effect of limiting the translation of most RNA transcripts in the cell. In the second pathway, introduction of long dsRNA catalyzes activation

of RNAse L, which degrades intracellular RNAs nonspecifically (Minks et al, 1979).

dsRNAs less than 30 base pairs in length, including siRNAs, do not appear to activate the PKR nonspecific response pathway in mature mammalian cells. Utilizing the knowledge that siRNAs can induce RNAi in *Drosophila* embryos, Elbashir and colleagues transiently transfected different mammalian cell cultures (Elbashir et al, 2001) and achieved as much as 90% reductions in target RNA levels. As in *Drosophila* and *C. elegans*, the effect was extremely sequence specific, with one base pair changes frequently being sufficient to abrogate silencing entirely. The design of siRNAs for use in mammalian cells is still a largely empirical process; there are apparently differential characteristics within genes, as yet unknown, that modulate the effectiveness of silencing by siRNAs localized to specific intragenic regions.

Most of the initial RNAi experiments in mammalian cells were carried out by tranfecting the cells (using a lipophilic carrier molecule such as Oligofectamine™) with chemically synthesized siRNAs. While this method has the advantage of initiating RNAi very quickly (usually within 18 hours), chemical synthesis of RNA is considerably more costly than DNA synthesis, and the induced RNAi lasts for a relatively short time. More recent approaches have included the transfection of RNA produced in T7 *in vitro* transcription reactions, which has the advantage of greatly reduced production costs but still has a relatively short half-life, and transfection of RNAi expression vectors, which can be stably integrated into the genome of the host cell and are theoretically capable

of providing transcriptional silencing of indefinite duration. Ambion Inc. has been at the forefront of efforts to produce and market such expression vectors. Their latest such products incorporate selectable markers into the vectors, allowing selection of successfully tranfected cells. This approach offers significant signal to noise ratio advantages when compared to other methods that result in production of a mixed population of cells, some successfully transfected and others not.

What is the role of RNA interference within a cell? Why has this function been so evolutionarily conserved through many different phyla? Phillip Sharp of MIT has been one of the leading proponents of the anti-viral hypothesis, which holds that RNAi is an ancient mechanism used by primitive eukaryotes to combat invading double stranded RNA viruses. Other theories, less prominent in the literature, cite RNAi as a possible defense against transposons or “jumping genes” and the deleterious effects they are capable of producing. In the attempts to identify the gene products required for RNAi in organisms as diverse as *Arabidopsis, Neurospora* and *C. elegans*, many researchers noted that such disruptions often resulted in organisms with severe errors in development, lending suppor to the idea that RNAi may also have a role to play in some aspects of development.

In summary, the history of the study of Parkinson's disease spans a relatively long period of time, with a dramatic increase in our knowledge of the disorder within only the last twenty years. The results that we outline in the following chapters verify the existence of mitochondrial-nuclear signaling in the context of electron transport chain deficits, as well as suggesting the vital roles played in this process by previously described intracellular signaling pathways. These results will serve to direct future investigations into gene expression changes relevant to the processes of cell death and cell survival in our cellular model of Parkinson's disease, and may provide important insights into the pathophysiology of the in vivo disease process.

Chapter 2

The first work that I undertook in the Center for the Study of Neurodegenerative Diseases involved the application of nylon membrane-based cDNA microarray technology to the issue of gene expression responses in a cellular model of Parkinson's disease, and concurrently investigated how these changes were dependent upon the energetic and genetic state of mitochondria and upon cell signaling pathways previously determined to modulate the cell death response under these conditions. The experiments were the basis for the publication "Dependence on electron transport chain function and intracellular signaling of genomic responses in SH-SY5Y cells to the mitochondrial neurotoxin MPP(+)", published in the journal *Experimental Neurology* (Brill and Bennett, 2003).

Summary

SH-SY5Y neuroblastoma cells exposed to the complex I inhibitor/parkinsonian neurotoxin methylpyridinium ion (MPP^+) activate both survival and death-promoting signaling pathways and undergo MEK/ERK-dependent, phosphatidylinositol-3 kinase-dependent and c-Jun kinase-dependent cell death. Because genomic responses to MPP^+ are not extensively characterized, we used nylon cDNA arrays to measured gene expression following exposure to an apoptosis-producing [MPP^+]. Many changes occurred within 5 minutes, and all gene expression changes appeared before biochemical

and morphological markers of apoptosis. Selective ablation of the mitochondrial genome of SY5Y cells through long-term exposure to low concentrations of ethidium bromide gives rise to a metabolically altered cell known as a ρ^0 (Cassarino et al., 1997; Cassarino et al., 2000; Swerdlow et al., 1996). These cells have no detectable mitochondrial DNA, and do not carry out oxidative phosphorylation. The majority of gene expression changes in SY5Y were not found in ρ^0 cells, indicating dependence of these changes on intact electron transport activity. ρ^0 cells exposed to MPP^+ produced different expression profiles, indicating the potential for responses independent of complex I inhibition. MPP^+-induced gene expression patterns in normal SY5Y cells were sensitive to inhibitors of MEK/ERK (U0126) or phosphatidylinositol-3 kinase (LY 294002), demonstrating regulation of gene expression by these survival-promoting signaling pathways. The primary signaling molecules mediating these MPP^+-induced gene expression changes are unknown but ultimately utilize MEK/ERK and phosphatidylinositol-3 kinase signaling. Genes suppressed by U0126 or LY294002 during MPP^+ exposure may mediate cell survival; those expressed in the presence of U0126 or LY294002 may mediate cell death in this *in vitro* model of Parkinson's disease.

Introduction

Among adults, Parkinson's disease (PD) is the most common neurodegenerative movement disorder and second most common

neurodegenerative brain disease. Motor deficits of PD arise from progressive loss of dopamine neurons in midbrain substantia nigra zona compacta, appear when ~60-75% of these neurons have died and progress towards severe disability as the remaining nigral dopamine neurons are lost at a rate of ~10% per year. While effective dopamine-replacement symptomatic treatments exist, none has been shown to change death rate of nigral neurons and alter disease outcome. Understanding how these dopamine neurons die may lead to neuroprotective therapies that will arrest progression of PD symptoms.

While the causes of neuronal cell death in PD remain elusive, significant insight arose in the mid 1980's when the mechanism of illness was defined in opiate addicts who had accidentally injected the pro-neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Ballard et al. 1985; Davis et al. 1979; Langston et al. 1983). These individuals developed rapidly progressive and severe parkinsonism responsive to dopamine replacement therapy. Animal studies showed that nigral dopamine neurons died following accumulation through the dopamine transporter of methylpyridinium ion (MPP^+), the two-electron monoamine oxidase oxidation product of MPTP and a potent inhibitor of complex I of the electron transport chain (Burns et al. 1983; Chiba et al. 1984; Jenner et al. 1984; Langston et al.1984; Javitch et al. 1985; Nicklas et al. 1985; Snyder and D'Amato 1986).

Because multiple PD tissues including platelets (Parker et al., 1989), muscle (Bindoff et al., 1991), fibroblasts (Mytilineau et al., 1994), and both nigral and nonigral brain (Mann et al., 1992; Mizuno et al., 1989) have reduced activity

of complex I, it has been suggested that PD represents a systemic complex I deficiency state that results in selective death primarily of nigral dopamine neurons. Such a concept was significantly strengthened by experiments with chronic systemic infusion into rats of the potent complex I inhibitor rotenone (Betarbet, et al, 2000). These animals developed a parkinsonian clinical condition and marked, selective loss of nigral dopamine neurons.

MPTP experiments in transgenic mice have demonstrated involvement of several gene products in neurotoxicity to dopamine neurons. Knockout of neuronal (Przedborski et al. 1996) or inducible (Dehmer et al. 2000) nitric oxide synthase, poly (ADP-ribose) polymerase (PARP, Mandir et al. 1999) or the growth regulatory gene p53 (Trimmer et al. 1996) reduces MPTP-induced loss of nigral dopamine neurons. These nigral dopamine neurons are protected against MPTP toxicity if the anti-apoptotic protein bcl-2 is overexpressed (Offen et al. 1998; Yang et al. 1998), or if the pro-apoptotic protein bax is knocked out (Vila et al. 2001).

Exposure of SH-SY5Y human neuroblastoma cells to MPP^+ induces apoptotic death (Fall and Bennett, 1999) and brings about many responses that likely regulate this process. These responses include an increase in oxidative stress (Cassarino et al., 1997), activation of pro- and anti-apoptotic signaling pathways (Cassarino et al., 2000; Halvorsen et al., 2002), and increasing levels of the anti-apoptotic proteins bcl-2 and bcl-X_L (Veech et al., 2000; Dennis and Bennett, 2002) and pro-apoptotic protein Bax in a nitric oxide-dependent manner (Dennis and Bennett, 2002). We have previously shown that MPP^+ induced

typical oligosomal DNA laddering in SY5Y cells, brought about DNA fragmentation measured with flow cytometry and increased reactive oxygen species (ROS) production and anaerobic metabolism, estimated by lactate production (Fall and Bennett, 1999).

Selective ablation of the mitochondrial genome of SY5Y cells through long-term exposure to low concentrations of ethidium bromide gives rise to a metabolically altered cell known as a ρ^0 (Cassarino et al., 1997; Cassarino et al., 2000; Swerdlow et al., 1996). Exposure of ρ^0 cells to 5 mM MPP^+ does not induce apoptotic cell death. We also found that ρ^0 cells exposed to 5 mM MPP^+ did not show increases in ROS or lactate production, demonstrating the necessity of intact electron transport chain function for MPP^+-induced increases in oxidative stress and anaerobic metabolism (Fall and Bennett, 1999).

We have examined the time course of cytochrome C release and caspase 3-dependent DNA fragmentation after exposure of SY5Y cells to MPP^+; cytochrome C appears in the cytoplasm within 2-4 hours, and caspase-dependent DNA degradation occurs between 12 and 24 hours (Dennis and Bennett, 2002). These findings suggest to us that acute complex I inhibition with MPP^+ may result in alteration of expression of multiple genes related to cell survival through signals as yet undetermined.

To explore this possibility, to determine if MPP^+-induced changes in gene expression depend on electron transport activity, and to explore what signaling pathways or molecules mediate these expression changes, we began this gene

expression study in SH-SY5Y cells exposed to MPP^+. We utilized cDNA arrays that displayed well-characterized genes related to cell death/survival and neuronal function. To achieve maximum sensitivity, we selected nylon-based cDNA arrays to which we hybridized ^{32}P-labeled probes. We wished to compare any changes in expression of this gene set to changes observed in SH-SY5Y cells devoid of mitochondrial DNA and electron transport activity (ρ^0 cells), cells treated with inhibitors of signaling pathways, other neurotoxins or neuroprotective drugs, and ultimately to postmortem PD brain samples.

Our results indicate that MPP^+ induces changes in expression of many different genes in SH-SY5Y cells. These gene expression changes occur hours before the appearance of biochemical and morphological markers of apoptosis. The majority of these changes are dependent on the presence of an intact mitochondrial genome and/or a functional electron transport chain (i.e., they are ablated in ρ^0 cells), and in addition are sensitive to inhibition of MEK or phosphatidylinositol-3-kinase (PI3K) signaling pathways. These data suggest that MPP^+ exerts significant effects on cells that factor in their commitment to apoptosis beyond inhibition of mitochondrial electron transport. Further, our results imply the existence of an efficient communication network between mitochondria and the nuclear genome, potentially permitting mitochondria to have more extensive control over cell functioning than previously thought.

Materials and Methods

Cell Culture and Treatment.

SH-SY5Y cells were obtained from the American Tissue Culture Collection (ATCC; Manassas, VA) and maintained in culture as previously described (Cassarino et al., 1997; Cassarino et al., 2000; Swerdlow et al., 1996). ρ^0 cells were generated by chronic incubation with ethidium bromide and maintained as previously described (Cassarino et al., 1997; Cassarino et al., 2000; Swerdlow et al., 1996). They had no mtDNA detectable by polymerase chain reaction and no electron transport chain activity based on assays of complex I and complex IV. 3 x 10^6 control or ρ^0 cells were placed onto 10 cm tissue culture plates (Greiner Bio-One; Longwood, FL) and maintained at 37°C for 24 hours prior to exposure to 5 mM MPP^+ (Sigma-Aldrich; St. Louis, MO) for multiple periods up to 3 hours in the presence or absence of 20 μM LY 294002 or 50 μM UO 126 for gene array hybridization studies. SH-SY5Y cells were exposed to 5 mM MPP^+ for multiple periods up to 24 hours for cell death studies.

RNA Isolation.

Total RNA was isolated from cell cultures immediately following completion of any MPP^+ exposure using the RNEasy Mini system (Qiagen; Valencia, CA). RNA samples were archived at -80°C.

cDNA Arrays.

Radioactively labeled cDNA probes were prepared from each total RNA samples and hybridized to Atlas nylon “Apoptosis” and “Neurobiology” arrays (Clontech Laboratories; Palo Alto, CA) as per kit protocol. α-^{32}P-dATP was obtained from Amersham Pharmacia Biotech, Piscataway, NJ. Array hybridization images were recorded in .TIF format using a Cyclone storage phosphor system (Packard Instrument Company; Meriden, CT). Exposure times were between 24 hours and 72 hours.

RT-PCR.

cDNA was prepared from experimental total RNA samples using the Advantage RT-for-PCR kit (Clontech Laboratories) using oligo(dT)primers according to the manufacturers directions. PCR was carried out using the Advantage cDNA PCR kit (Clontech Laboratories), with the indicated Atlas PCR primers according to manufacturers directions. Samples were held at 94^{o}C for 1 minute, followed by 33 cycles of [94^{o}C-30 seconds, 68^{o}C-2 minutes], followed in turn by a final 68^{o}C for 5 minutes. PCR products were separated on 2% agarose gels and visualized by ethidium bromide incorporation. Caspase 7 and NIK were selected for RT-PCR verification of both statistically significant and non-significant gene expression changes.

Statistical Analysis.

Three independent cell culture, RNA extraction and array hybridization experiments were performed for each period of exposure to MPP^+. Optical density and background levels for each gene were extracted from TIFF files using ImaGene software (BioDiscovery; Los Angeles, CA). Optical densities for individual genes were normalized to the mean optical density calculated from hybridization signals for 40S ribosomal protein, 60S ribosomal protein and cytoplasmic β-actin "housekeeping genes" in each array experiment, allowing direct comparisons across arrays. When normalization was performed on the basis of combined densities for all genes present on each array, signals were found to be no greater than 1% different from those obtained through housekeeping gene normalization. Normalized gene hybridization signals of at least 0.1 relative intensity were analyzed by cluster analysis using Cluster software and results visualized using TreeView software (both from M. Eisen, Lawrence Berkeley National Laboratory, http://rana.lbl.gov). One-way repeated measures ANOVA was used to evaluate differences in gene hybridization signals across time of MPP^+ exposure. Two-way repeated measures ANOVA was used to compare gene hybridization signals across time of MPP^+ exposure and cell type when comparing SY5Y to ρ^0. P values less than 0.05 were considered significant.

Results

SY5Y Response to MPP^+

Our approach to this gene expression study was to use the most sensitive array detection technology (^{32}P-labeled cDNA oligonucleotide probes) and multiple, independent (n=3) experiments for each condition to control for biological and methodological variations. Cluster analysis provides a non-weighted, grouping paradigm that allows deliniation of gene groups whose relative expression levels change with similar patterns. Output of the Cluster software program is presented in Figure 1; a branching dendrogram indicating the grouping of the genes and a corresponding graphical representation of each gene expression profile is shown. Analysis of the four second-order nodes identified by the program indicated marked differences in temporal gene expression patterns among these clusters. Cluster 1, the smallest of the four, identified genes (n=18) with a punctuated decrease in expression at the 15 minute timepoint, and little significant regulation at other timepoints. Cluster 2 identified a large group of genes (n=171) characterized by an even earlier decrease in expression, at the 5 minute timepoint. Some genes in this cluster had little other significant regulation, whereas others were consistently downregulated throughout the time course; others still are notable for slight upregulation of expression at the 90 minute timepoint. Cluster 3 represents genes (n=83) whose expression was upregulated early in the time course, the majority of which have their peaks of expression at the 30 minute timepoint. The

fourth and final cluster contains genes (n=106) that are upregulated at the later (90 minute plus) points in the timecourse, with considerable heterogeneity of expression at the earlier timepoints. The complete listing of genes for each mathematical cluster is provided in the Supplemental Data section.

We used parametric ANOVA testing to search for any significant variation in expression of individual genes. 36 individual genes were found to be significantly regulated during MPP^+ exposure. A listing of these genes is presented in Table 1, part A.

SY5Y ρ^0 Response to MPP^+

In order to investigate the contribution of electron transport chain activity to the cellular genomic response to MPP^+, we collected expression data from SY5Y ρ^0 cultures exposed in triplicate to 5 mM MPP^+ for 0, 15, 60 and 120 minutes. and compared these to expression profiles of control SY5Y exposed to MPP^+ for identical time periods. Expression profiles of genes detected in both SY5Y and SY5Y ρ^0 cells are compared by cell type in Figure 2. Of the 378 genes detected in SY5Y cells, 261 were also detected in SY5Y ρ^0 cells. There were 20 genes detected in SY5Y ρ^0 cells that were not detected in control SY5Ys. For the 261 in-common genes, data from the time points measured in ρ^0 cells were compared to equivalent data from control cells by 2-way ANOVA and found to be significantly different ($P < 0.05$). Furthermore, of the 261 in-common

genes, 51 were found to be regulated in opposite directions at all timepoints measured in control and ρ^0 SY5Y cells.

17 individual genes were found to be significantly regulated in ρ^0 cells during MPP^+ exposure. A listing of these genes is presented in Table 1, part B. Note that only 2 out of 770 genes detected across both cell types, furin and GADD153, were found to be significantly regulated in both cell types.

SY5Y Response to MPP^+ in the presence of LY 294002 and UO 126

Previous results (Halvorsen, et al, 2002) indicated that inhibition of PI3-kinase or ERK signaling pathways accelerated MPP^+ induced cell death in SH-SY5Y cells. We therefore analyzed gene expression in SH-SY5Y cells exposed to LY 294002 (LY) and UO 126 (UO), pharmacological inhibitors of PI3-kinase and ERK signaling, respectively, to determine what changes in gene expression might be involved in commitment to cell death. LY 294002 is a reversible, specific inhibitor of PI3-kinase that competes for ATP binding on PI3-kinase; its IC50 for class I PI3-kinases is 1 μM and for class II PI3-kinases is 19 μM (Vlahos, et al, 1994). UO 126 is a specific inhibitor of MEK1 and MEK2; it inhibits phosphorylation-activated as well of constitutively active forms of both MEK1 and MEK2, with IC_{50} values of 10 μM and < 0.1 μM, respectively (Favata, et al 1998). We found distinct groups of genes that were significantly regulated during exposure to these compounds; a total of 22 individual genes modulated by LY treatment are summarized in Table 2 part A. 51 genes modulated by UO

treatment are summarized in Table 2 part B. 7 genes out of 76 significantly regulated by either LY or UO plus MPP^+ were also significantly regulated by MPP^+ alone. 4 genes are significantly regulated by both drugs in the presence of MPP^+.

To estimate the effect of LY and UO on baseline gene expression, we compared expression data from control SH-SY5Y cells and SY5Ys exposed to either LY or UO alone. Results are presented in Figure 3. Baseline expression was highly similar for both LY and UO when compared to control, with a slight overall tendency toward reduced expression (note relative positions of regression lines in both scatterplots to equivalence lines).

RT-PCR

We sought to validate the gene array data in terms of gene expressions that change and those that don't change. PCR primers specific for arrayed genes caspase 7 and NIK were obtained from Clontech laboratories. RT-PCR was optimized and performed using RNA from control SY5Y cells and SY5Ys treated with 5 mM MPP^+ for 90 minutes. We examined the caspase 7 gene because it codes for an executioner caspase and had a low level of increase after MPP^+ (1.5-fold, NS). RT-PCR showed an increase in caspase-7 mRNA of 1.3-fold in the same samples. The NIK gene exhibited one of the largest increases after MPP^+ (see Table 1A). At 90 minutes of MPP^+ treatment the gene arrays showed an increase in NIK expression of 5.8-fold. RT-PCR assay of the same samples showed an increase of 6.0-fold. Thus, RT-PCR analysis agreed with array data

for genes with both a low, non-significant level of increase, and a high level of increase.

Discussion

MPTP treatment of animals and MPP^+ exposure of neural cells have been extensively utilized as models of neuronal death for Parkinson's disease. Prior characterizations of the effects of the parkinsonism-producing protoxin MPTP and its toxic metabolite MPP^+ at the cellular level have focused on the role of MPP^+ as an inhibitor of complex I of the mitochondrial electron transport chain (ETC). The increased production of ROS resulting from shunting of electrons away from their normal acceptor molecules as a result of this ETC inhibition has been suggested as a possible mediator of the damage to cellular components and eventual progression to apoptotic cell death that follows exposure to MPP^+. Our experiments utilized a focused genomics approach to address interrelated questions about MPP^+ toxicity. First, we wished to determine the changes in gene expression that occur in cells in response to MPP^+ exposure prior to apoptosis. Second, by utilizing genomic responses to MPP^+ in ρ^0 cells, we explored the involvement of the ETC in mediating observed expression changes in native SH-SY5Y neuroblastoma cells. Finally, we utilized genomic response screening by array in combination with pharmacological manipulation of signaling pathways known to be contributory to MPP^+ induced cell death, with an objective of determining which pathways may be responsible for initiating or preventing cell death.

The statistical analysis of microarray data is still far from standardized, and the relative merits of multiple methods are matters of active discourse. Further, it remains unknown if there are any correlations among mRNA changes and alterations in protein levels in this or many other cell models. With this in mind, we adopted a conservative, parametric analysis strategy that relied upon experimental replication and well-characterized statistical tests. We have listed all gene changes that achieved statistical significance and have not assigned an arbitrary minimum value of "biological significance." Having proceeded in this manner, we believe that the results presented here are a minimal listing of genomic responses, and the reader should not assume that additional, significant genomic responses do not or cannot occur under similar experimental conditions.

The very rapid appearance of gene expression changes observed in SY5Y cells exposed to MPP^+ was surprising. As our studies of cell death markers in the SY5Y-MPP^+ model had found that the earliest markers of apoptosis appeared in the 2-4 hour time range, it was interesting to note that significant alterations in gene expression occurred within 5-15 minutes of MPP^+ exposure. While this does not mean that any of these alterations in expression are required for apoptosis to occur, it is unlikely that all the observed changes are unrelated to the process of apoptosis. Given that the criteria deciding commitment to apoptosis are incompletely defined, it is possible that the results presented here will eventually lead to a more robust depiction of the process of programmed cell death that might allow for earlier identification of cells fated for apoptosis, perhaps by observing changes in genomic expression.

These studies were undertaken with an initial objective of monitoring the changes in expression of genes known to be involved in apoptosis, e.g. caspases, bcl family proteins, etc., as well as those important to broader mitochondrial functions, e.g. porin, nitric oxide synthase, etc. The diversity of gene responses induced by MPP^+ treatment is difficult to overstate, ranging from cyclin-dependent kinases required for mitosis to the workaday enzymes of basic cellular metabolism. As such, the little-understood effects of MPP^+ on cellular components other than mitochondria have assumed a new importance in our considerations for future experiments.

We found that the expression level of 378 genes could be reliably detected across the period of MPP^+ exposure in control and ρ^0 SY5Y cells combined. Of these genes, expression levels for 261 were present in ρ^0 samples. ρ^0 cells have no detectable mtDNA and no measurable ETC activity, therefore compounds that inhibit ETC activity are unlikely to produce ROS through conventional mitochondrial mechanisms in ρ^0 cells. In previous work we found that SH-SY5Y ρ^0 cells neither undergo apoptosis after extended exposure to 5 mM MPP^+, nor produce detectable increases in ROS or increases in glycolytic metabolism after MPP^+ exposure (Fall and Bennett, 1999). Thus, the changes in gene expression observed in ρ^0 cells treated with MPP^+ are unlikely to be mediated by increases in oxidative stress or intracellular acidification. Given that MPP^+ is regarded primarily as an inhibitor of the ETC, it was quite remarkable to note that greater than two-thirds of gene expression changes that

occur following treatment with MPP^+ are likely to be completely unrelated to this aspect of its toxicity; the logical corollary to this idea is that those gene changes observed in SY5Y but not ρ^0 cells following MPP^+ treatment are in some way dependent on ETC function.

We found that the expression of two genes, furin and GADD153 were significantly regulated in both SY5Y and ρ^0 cells. Furin is a Ca(2+)-dependent serine endoprotease that belongs to the subtilisin-like proprotein convertase (SPC) family. Furin has been shown to be expressed in all tissues and cell lines examined and to be mainly localized in the *trans*-Golgi network, although some proportion of the furin molecules cycle between this compartment and the cell surface. This endoprotease is capable of cleaving precursors of a wide variety of proteins, including growth factors, serum proteins, including proteases of the blood-clotting and complement systems, matrix metalloproteinases, receptors, viral-envelope glycoproteins and bacterial exotoxins (Nakayama, 1997). Furin has recently been implicated in the extracellular cleavage of proneurotrophins, including proNGF. This regulatory control is particularly interesting in the light of evidence that proneurotrophins stimulate apoptosis in target cells, whereas the proteolyzed, mature forms promote nerve cell survival (Lee, et al, 2001).

Growth arrest- and DNA damage-inducible gene 153 (GADD153), a leucine-zipper transcription factor which is part of the CCAAT/enhancer-binding protein (C/EBP) family, is also known as CHOP (C/EBP homologous protein). It is not expressed at detectable levels in growing mammalian cells but is strongly upregulated in response to various cellular insults (Fawcett, et al, 1996; Luethy

and Holbrook, 1992). Recent experiments have shown a functional link between GADD153 expression and cellular alterations including growth arrest, apoptosis and even tumorigenesis. Injection of GADD153 protein into NIH3T3 cells caused G1/S arrest of the cell cycle (Barone, et al, 1994). In contrast, overexpression of GADD153 in M1 myeloblastic leukemia cells induced apoptosis, which can be modified by the anti-apoptotic protein, Bcl-2 (Matsumoto, et al, 1996). In addition, compared to the wild type, mouse embryonic fibroblasts derived from gadd153$^{-/-}$ knockout animals undergo significantly less apoptosis in response to endoplasmic reticulum (ER) stress, a powerful upregulator of GADD153 expression (Zinszner, et al, 1998). GADD153 induction has been shown to be required for apoptosis in a human colon cancer cell line induced by treatment with bile acids (Qiao, et al, 2002). Recently, Conn ,et al (2002) reported that treatment of SY5Y cells with 1 mM MPP^+ differentially altered expression of 313 genes after 24 hours, and that GADD153 expression increased the most. They also found a modest increase in GADD153 protein by Western blot at 24 hours, with protein levels declining after 24 hours in spite of continued increase in mRNA.

Our findings may provide an example of the interplay of pro- and anti-apoptotic elements within cells exposed to MPP+; as furin expression was reduced and GADD153 was increased in normal SY5Y cells in response to MPP^+, this may indicate anti-apoptotic and pro-apoptotic roles for these gene products, respectively, with MPP^+ exposure directing the balance toward cell death. The changes observed in ρ^0 cells are similar to those in control SY5Y

cells (furin down, GADD153 up); taken together, these results may suggest that these expression changes are necessary but not sufficient for apoptosis, as ρ^0 cells do not undergo apoptosis in response to 5 mM MPP^+ (Fall and Bennett, 1999).

How might MPP+ bring about gene expression changes in ρ^0 cells devoid of ETC function? An additional non-ETC action for MPP^+ is its recently described ability to open a traditional mitochondrial transition pore (Cassarino, et al, 1999). This transition pore opening was observed in liver mitochondria to occur synergistically with nitric oxide and was incompletely inhibited by ROS scavenging enzymes, suggesting a possible direct interaction of MPP^+ with transition pore components. This concept is supported by our observation that ρ^0 cells can be induced to undergo caspase 3-mediated chromatin cleavage into DNA-histone complexes, if metabolic substrate for ATP production (pyruvate) is supplied (Dennis and Bennett, under review). An additional consideration is that MPP^+-induced genomic alterations we observed in ρ^0 cells may wholly or partly derive from non-mitochondrial actions of MPP^+. Whatever the final explanations are, MPP^+ is clearly capable of inducing multiple cellular genomic responses beyond the purview of its known ETC inhibitory actions. This observation raises the problem of how MPP^+ effects in cells and brain are to be interpreted. Assuming that biochemical changes or cell death in MPTP/MPP^+ experiments derive exclusively from ETC inhibition is no longer viable.

When compared to the number of genes detected in SY5Y and ρ^0 cells, only a small portion, ~9% (35/378), were found to change significantly over the time course of MPP^+ exposure. When this set of genes is compared to similar sets derived from ρ^0 cells or from SY5Y cells exposed to inhibitors of the MEK and/or PI3K pathways, we note a very small number of genes in common. While it is not surprising that significant differences exist between the datasets, particularly between ρ^0 cells and SY5Ys, the degree of dissimilarity is striking; only 7 genes out of 76 significantly regulated by either LY or UO plus MPP^+ were also significantly regulated by MPP^+ alone (Table 4 parts A and B). This high degree of dissimilarity between the sets of genes regulated by LY or UO plus MPP^+ and those regulated by MPP^+ alone compels us to consider the possibility that cellular responses, mitochondrial and otherwise, to MPP^+ are in some way dependent upon ERK and/or PI3K activity. Furthermore, there are only 4 genes (2 of which encode lysosomal proteins) that are significantly regulated by both drugs in the presence of MPP^+ (Table 4 part C).

In only one gene, LIMK-1, was expression increased by MPP+ alone and decreased by MPP+ in combination with an inhibitor (UO 126). While this nonconcordance of response is consistent with LIMK-1 performing a necessary cell survival function in response to MPP+ insult, its connection with known apoptosis/survival mechanisms is unclear. It is a serine threonine protein kinase remarkable for its 2 N-terminal LIM domains that are highly conserved cysteine-rich structures containing 2 zinc fingers thought to be responsible for the

specificity of its protein-protein interactions. Its best characterized cellular function is the transduction of phosphorylation changes in Rho protein to changes in the state of the actin cytoskeleton via phosphorylation of cofilin (CFL1) (Maekawa, et al, 1999). Additionally, LIMK-1 hemizygosity has been associated with Williams syndrome, a developmental disorder in which patients suffer from poor visuospatial constructive cognition (Frangiskakis, et al, 1996). As previous work in our laboratory has documented the ability of inhibition of MEK and PI3K to accelerate progression to cell death in the presence of MPP^+, these data suggest the possibility that these 4 genes or related gene products may be involved in mediating cell death induced by MPP^+. These findings also serve to provide direction for our future inquiries, particularly the question of whether the sets of genes observed to change significantly in the presence of either inhibitor in addition to MPP^+ represent bona fide independent pathways leading to apoptosis, or act under control of other unidentified cell signaling components.

A potential limitation of our study is that we utilized SH-SY5Y neuroblastoma cells exposed to a high [MPP^+]. SH-SY5Y cells expressing the dopamine transporter (DAT) and exposed to 10 μM [MPP+] showed acute mitochondrial impairment and delayed (2-3 days) apoptotic death (Stephans, et al, 2002). This finding suggests that it is the low level of DAT expression in native SH-SY5Y that requires high [MPP^+] for induction of apoptosis in our paradigm. We chose to use this paradigm in order to be able to compare gene expression profiles with our earlier studies in SH-SY5Y cells on the rate and characteristics

of apoptotic cell death (Fall and Bennett, 1999), activation of intracellular signaling (Cassarino, et al, 2000; Halvorsen, et al, 2002) and NF-kB transcription factor (Cassarino, et al, 2000), release of cytochrome C and activation of caspases (Abramova, et al, 2002) and changes in mRNA and protein levels for apoptosis-regulating bcl-family proteins (Veech, et al, 2000; Dennis and Bennett, 2002). However, we note that because the nature of cell death in Parkinson's disease remains controversial (Graeber and Moran, 2002), our use of acute, MPP^+-induced apoptosis in undifferentiated, dividing neuroblastoma cells may be of limited relevance to neurodegeneration in this clinical disease.

Finally, our findings demonstrate an extensive and rapidly acting signaling system between mitochondrial "distress" and nuclear gene expression. We do not yet know which molecules or signaling pathways mediate these expression changes, but it is likely that such signaling is necessary for cell survival in the face of mitochondrial insults or failure. Because increasing evidence points to mitochondrial impairments as a leading cause of neurodegeneration (Cassarino and Bennett, 1999), elucidating the signaling mechanisms between mitochondria and the nuclear genetic machinery of their host cells could point to additional strategies to increase neuronal survival in these chronic, progressive diseases.

Chapter 2 Table 1.
A. Genes significantly regulated in SH-SY5Y exposed to MPP^+ (n=36)

Gene	Chromosomal Location	Genbank #	Max fold change from baseline	p-value
94-kDa glucose-regulated protein (GRP94)	12q24.2-12q24.3	X15187	-0.83	0.020
phosphatidylinositol 4-kinase alpha (PI4-kinase; PTDINS-4-kinase; PI4K-alpha)	22q11.21	L36151	-0.82	0.007
fur; furin precursor; paired basic amino acid residue cleaving enzyme (PACE); dibasic processing enzyme*	15q25-15q26	X17094	-0.81	0.005
p21-activated kinase alpha (PAK-alpha; PAK1)	11q13-11q14	U24152	-0.81	0.034
lumican precursor (LUM); keratan sulfate proteoglycan; LDC	12q21.3-12q22	U18728	-0.79	0.021
protein-tyrosine phosphatase 2C (PTP-2C); SH-PTP2	12q24.1	L08807	-0.74	0.028
cAMP-dependent protein kinase alpha-catalytic subunit (PKA C-alpha)	19p13.1	X07767	-0.70	0.010
phosphatidylethanolamine-binding protein (PBP); neuropolypeptide H3	12q24.23	D16111	-0.71	0.013
Ras-related protein RAB-5B	12q13	X54871	-0.69	0.033
mitochondrial ATP synthase alpha chain precursor; ATP5A1	18q12-q21	D14710	-0.68	0.019
casein kinase I alpha isoform (CKI-alpha); CK1; CSNK1A	13q13	X80693	-0.66	0.046
Ras-related protein RAB-32	6q24.3	U71127	-0.65	0.048
ephrin type-A receptor 1 precursor; tyrosine-protein kinase receptor eph	17q32-17q36	M18391	-0.65	0.025
outer mitochondrial membrane protein porin; porin 31HL; porin 31HM	5q31	L06132	-0.64	0.045
beta-adrenergic receptor kinase 2 (beta-ARK2)	22q11	X69117	-0.63	0.006
laminin beta 1 subunit precursor	7q31.1-7q31.3	M61916	-0.61	0.044

(laminin B1; LAMB1)				
casein kinase I delta isoform (CKI-delta); CSNK1D	17q25	U29171	-0.61	0.016
insulin-like growth factor II receptor (IGFR II)	11p15.5	Y00285	-0.61	0.006
metabotropic glutamate receptor 7 precursor (GRM7; MGLUR7)	3p26.1-p25.1	X94552	-0.60	0.038
cholinergic receptor nicotinic alpha polipeptide 3 (CHRNA3)	2q24-2q32	M37981	-0.59	0.012
Ras-related protein RAB-11A; YPT3	15q21.3-q22.31	X53143	-0.59	0.037
sodium-dependent noradrenaline transporter; norepinephrine transporter (NET)	16q12.2	M65105	-0.59	0.030
protein-tyrosine phosphatase mu precursor (R-PTP-mu)	18p11.2	X58288	-0.57	0.025
lysosomal acid phosphatase precursor (LAP); ACP2	11p11.2	X12548	-0.56	0.035
MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)	1q32	U12779	-0.56	0.024
vesicular acetylcholine transporter (VAChT)	10q11.2	U10554	-0.51	0.041
LIM domain kinase 1 (LIMK-1)	7q11.23	D26309	0.68	<0.001
protein kinase C inhibitor protein-1 (KCIP-1)	20q13.1	X57346	0.79	0.031
Ras-related protein RAB5A	3p24-p22	M28215	0.97	0.023
RBQ1 retinoplastoma binding protein	16p12-p11.2	X85133	0.98	0.035
serine/threonine protein phosphatase PP1-gamma 1 catalytic subunit (PP-1G)	12q24.1-q24.2	X74008	1.0	0.025
transforming protein rhoA H12 (RHO12; ARH12; ARHA)	3p21.3	L25080	1.2	0.045
growth arrest & DNA-damage-inducible protein 153 (GADD153)*	12q13.1-12q13.2	S40706	1.3	0.028
G1/S-specific cyclin D1 (CCND1); cyclin PRAD1; bcl-1 oncogene	11q13	X59798	1.5	0.024
NIK serine/threonine protein kinase	17q21	Y10256	9.4	0.039

cell division protein kinase 3	17q22	X66357	11.	0.027

B. Genes significantly regulated in SH-SY5Y ρ^0 exposed to MPP^+ (n=17)

Gene	*Chromosomal Location*	*Genbank #*	*Max fold change from baseline*	*p-value*
endothelial nitric oxide synthase (EC-NOS; ENOS); type III NOS	*7q36*	*M93718*	*-11*	*0.011*
adenosine A1 receptor (ADORA1)	*1q32.1*	*S56143*	*-7.4*	*0.005*
apolipoprotein E precursor (APOE)	*19q13.2*	*M12529*	*-2.6*	*0.018*
corticotropin releasing factor receptor 1 precursor (CRF-R; CRF1)	*17q12-q22*	*X72304*	*-1.9*	*<0.001*
*Fur; furin precursor; paired basic amino acid residue cleaving enzyme (PACE); dibasic processing enzyme**	*15q25-15q26*	*X17094*	*-1.9*	*0.014*
ER-Golgi intermediate compartment 53-kDa protein (ERGIC-53); GP58; MR60; mannose-binding lectin (LMAN1)	*18q21.3-q22*	*X71661*	*-1.5*	*0.006*
superior cervical ganglion-10 protein (SCG10); neuron-specific growth-associated protein; stathmin homolog	*8q21.12*	*S82024*	*-1.2*	*0.004*
annexin II (ANX2); lipocortin II; calpactin I heavy subunit; chromobindin 8; protein I; placental anticoagulant protein IV (PAPIV)	*15q21-q22*	*D00017*	*-1.1*	*0.037*
protein kinase C alpha polypeptide (PKC-alpha; PKCA)	*17q22-q23.2*	*M22199*	*-1.1*	*0.028*

Calmodulin	*19q13.2-q13.3*	*J04046*	*-1.1*	*0.022*
beta-D-galactosidase precursor; lactase; acid beta-galactosidase; GLB1	*3p21.33*	*M27507*	*0.50*	*0.013*
drebrin E	*5q35.3*	*U00802*	*0.53*	*0.047*
serine/threonine protein phosphatase PP2A-beta catalytic subunit	*8p12-p11.2*	*X12656*	*0.55*	*0.002*
induced myeloid leukemia cell differentiation protein MCL-1	*1q21*	*L08246*	*0.58*	*0.019*
c-jun proto-oncogene; transcription factor AP-1	*1p32-p31*	*J04111*	*0.64*	*0.018*
dopamine beta-hydroxylase (DBH); dopamine-beta-monooxygenase precursor	*9q34*	*X13255*	*0.68*	*0.015*
*growth arrest & DNA-damage-inducible protein 153 (GADD153)**	*12q13.1-12q13.2*	*S40706*	*0.83*	*0.007*

Table 1 Legend

** denotes genes significantly regulated in both cell types.*

"Max fold change from baseline" refers to the maximum change in signal observed for a given gene over all time points examined as compared to average (mean) signal observed in control cells. The time point of maximal change in expression varies from gene to gene.

Chapter 2 Table 2.

A. Genes significantly regulated in SH-SY5Y exposed to LY 294002 and MPP^+ (n=22)

Gene	Location	Genbank #	Max fold change from baseline	p-value
cyclin-dependent kinase 4 inhibitor (CDK4I; CDKN2); p16-INK4; multiple tumor suppressor 1 (MTS1)	9p21	L27211	-0.59	0.045
ras-related protein RAB-32*	6q24.3	U71127	-0.34	0.009
protein phosphatase PP2A 55-kDa regulatory subunit neuronal isoform; protein phosphatase PP2A B subunit beta; beta-PR55†	5q31-5q33	M64930	-0.25	0.004
cyclophilin 3 protein (CYP3); mitochondrial peptidyl-prolyl cis-trans isomerase precursor (PPIASE); rotamase	10q22-q23	M80254	-0.23	0.037
serine/threonine-protein kinase PCTAIRE 1 (PCTK1)	Xp11.3-p11.23	X66363	-0.22	0.032
cyclin-dependent kinase 4 inhibitor D (CDKN2D); p19-INK4D	19p13	U40343	-0.22	0.025
cation-dependent mannose-6-phosphate receptor precursor (CD man-6-P receptor; CD-MPR); MPR 46	12p13	M16985	-0.19	0.005
syntaxin 7 (STX7)	6q23.1	U77942	-0.17	0.030
c-myc binding protein MM-1	12q13.13	D89667	-0.14	0.022
dystroglycan precursor; dystrophin-associated glycoprotein 1 (DAG1)	3p21	L19711	-0.07	0.029
MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)*	1q32	U12779	-0.02	0.015
protein-tyrosine phosphatase 1B (PTP-1B)	20q13.1-q13.2	M31724	0.13	0.008
activin receptor type IIB (ACTRIIB; ACVR2)	3p22	X77533	0.20	0.022
protein SEC23 homolog isoform A (SEC23A)	14q13.2	X97064	0.24	0.005
MAPK/ERK kinase kinase 3 (MEK	17q11.2	L36719	0.26	0.018

kinase 3; MEKK3)				
alpha-soluble NSF attachment protein (SNAP-alpha)	19q13.33	U39412	0.31	0.010
Rac-alpha serine/threonine kinase (rac-PK-alpha); protein kinase B (PKB); c-akt; akt1	14q32.32	M63167	0.43	0.027
cytosolic dynein heavy chain (DYHC)	14q32.3	L23958	0.44	0.012
lysosomal acid lipase/cholesteryl ester hydrolase precursor (LAL); acid cholesteryl ester hydrolase; sterol esterase; lipase A (LIPA); cholesteryl esterase†	10q23.2-q23.3	M74775	0.50	0.015
lysosome membrane protein II (LIMP II); 85-kDa lysosomal membrane sialoglycoprotein (LGP85); CD36 antigen-like 2 (CD36L2)†	4q21.21	D12676	0.53	0.012
insulin-like growth factor-binding protein 3 precursor (IGF-binding protein 3; IGFBP3; IBP3)†	7p13-p12	M31159	0.66	0.015
neuron-derived orphan receptor 1 (NOR1); mitogen-induced nuclear orphan receptor (MINOR); CHN	9q22	D78579	0.80	0.025

B. Genes significantly regulated in SH-SY5Y exposed to MPP^+ and UO 126 (n=51)

Gene	Location	Genbank #	Max fold change from baseline	p-value
chromaffin granule amine transporter; vesicular amine transporter 1 (VAT1)	8p21.3	U39905	-0.98	0.005
ER lumen protein retaining receptor 1; KDEL receptor 1; ERD 21	19q13.3	X55885	-0.91	0.023
mitochondrial 10-kDa heat shock protein (HSP10); 10-kDa chaperonin (CPN10); HSPE1	2q33.1	U07550	-0.84	0.018
protein phosphatase PP2A 55-kDa regulatory subunit alpha isoform; protein phosphatase PP2A B subunit alpha isoform; alpha-PR55†	8p21.1	M64929	-0.79	0.042
neural-cadherin precursor (N-cadherin; NCAD); cadherin 2 (CDH2)	18q11.2	M34064	-0.76	0.001
lumican precursor (LUM); keratan sulfate proteoglycan; LDC*	12q21.3-q22	U18728	-0.75	0.041
LIM domain kinase 1 (LIMK-1)*	7q11.23	D26309	-0.74	0.032
serine/threonine protein phosphatase PP1-gamma 1 catalytic subunit (PP-1G)	12q24.1-q24.2	X74008	-0.72	0.036
paraneoplastic encephalomyelitis antigen HUD; HU-antigen D	1p34	M62843	-0.71	0.039
epidermal growth factor receptor kinase substrate EPS8	12q23-q24	U12535	-0.68	0.038
dihydropyridine-sensitive L-type calcium channel beta-3 subunit (CAB3A/CAB3B); CACNLB3	12q13	U07139	-0.65	0.040
lung group IB phospholipase A2 precursor (PLA2); phosphatidylcholine 2-acylhydrolase	12q23-q24.1	M21054	-0.65	0.034
mitochondrial stress-70 protein precursor; 75-kDa glucose-regulated protein (GRP75); peptide-binding protein 74 (PBP74); mortalin (MOT); HSPA9B	5q31.1	L15189	-0.63	0.022
flavoprotein subunit of complex II;	5p15	D30648	-0.62	0.049

succinate-ubiquinone dehydrogenase flavoprotein subunit precursor (SDHA; SDH2)				
coatomer alpha subunit; alpha-coat protein; alpha-COP; HEP-COP	1q23-q25	U24105	-0.62	0.021
94-kDa glucose-regulated protein (GRP94)*	12q24.2-12q24.3	X15187	-0.60	0.002
casein kinase II alpha' subunit (CK II); CSNK2A2	16p13.3-p13.2	M55268	-0.58	0.043
Golgi SNARE; GS27	17q21	AF007548	-0.55	0.014
lysosome membrane protein II (LIMP II); 85-kDa lysosomal membrane sialoglycoprotein (LGP85); CD36 antigen-like 2 (CD36L2)†	4q21.21	D12676	-0.53	0.022
ras-related protein R-ras2; ras-like protein TC21; teratocarcinoma oncogene	11p15.3	M31468	-0.51	0.019
vesicle-membrane fusion protein SNAP23A	15q13.3	Y09567	-0.49	0.021
cholinephosphate cytidylyltransferase; phosphorylcholine transferase; CTP	3q29	L28957	-0.48	0.023
protein-tyrosine phosphatase MEG2 (PTPASE-MEG2)	15q22.9	M83738	-0.46	0.047
laminin gamma 1 subunit precursor (LAMC1); laminin B2 subunit (LAMB2)	1q31	J03202	-0.45	0.032
lysosomal acid lipase/cholesteryl ester hydrolase precursor (LAL); acid cholesteryl ester hydrolase; sterol esterase; lipase A (LIPA); cholesteryl esterase†	10q23.2-q23.3	M74775	-0.43	0.049
ER-Golgi intermediate compartment 53-kDa protein (ERGIC-53); GP58; MR60; mannose-binding lectin (LMAN1)	18q21.3-q22	X71661	-0.42	0.017
guanine nucleotide-binding protein beta subunit-like protein 12; receptor of activated protein kinase C 1 (RACK1)	5q35.3	M24194	-0.40	0.017
p53 cellular tumor antigen	17p13.1	M14694	-0.39	0.019
casein kinase I alpha isoform (CKI-alpha); CK1; CSNK1A*	13q13	X80693	-0.33	0.047
membrane-bound & soluble	22q11.21	M65212	-0.29	0.017

catechol-O-methyltransferase (COMT)				
protein-tyrosine phosphatase D1 (PTP-D1)	14q31.3	X79510	-0.26	0.024
cell division protein kinase 6 (CDK6); serine/threonine protein kinase PLSTIRE	7q21-q22	X66365	0.43	0.046
E2F dimerization partner 1; DRTF1-polypeptide 1 (DP1)	13q34	L23959	0.44	0.047
p73 (monoallelically expressed p53-related protein)	1p36.3	Y11416	0.45	0.014
cell division cycle protein 25A (CDC25A); M-phase inducer phosphatase 1	3p21	M81933	0.88	0.034
CDC27HS protein	17q12-17q23.2	U00001	0.88	0.034
p35 cyclin-like CAK1-associated protein	14q23	X92669	0.92	0.046
RBQ1 retinoplastoma binding protein*	16p12-p11.2	X85133	1.0	0.042
RBQ-3	1q32	X85134	1.0	0.015
insulin-like growth factor-binding protein 3 precursor (IGF-binding protein 3; IGFBP3; IBP3)†	7p13-p12	M31159	1.2	0.033
Peptidyl-prolyl cis-transisomerase nima-interacting 1 (PIN1)	19p13	U49070	1.3	0.018
NEDD5 protein homolog; DIFF6; KIAA0158	2q37	D63878	1.3	0.026
glutathione S-transferase mu1 (GSTM1; GST1); HB subunit 4; GTH4	1p13.3	X08020	1.4	0.032
PDCD2	6q27	S78085	1.4	0.027
Ubiquitin-conjugating enzyme E2 32-kDa complementing protein; ubiquitin-protein ligase; ubiquitin carrier protein; CDC34	19p13.3	L22005	1.7	0.047
glutathione S-transferase A1 (GTH1; GSTA1); HA subunit 1; GST-epsilon	6p12.2	M25627	1.9	0.011
Wee1Hu CDK tyrosine 15-kinase; wee-1-like protein kinase	11p15.3-p15.1	U10564	2.0	0.039
CDC10 protein homolog	7p14.3-p14.1	S72008	2.3	0.030
TNF-alpha converting enzyme (TACEA); transmembrane	2p25	U69611	2.7	0.015

metalloproteinase/disintegrin; adamalysin				
c-raf proto-oncogene	3p25	X03484	3.3	0.032
G2/mitotic-specific cyclin B1 (CCNB1)	5q12	M25753	3.6	0.037

Table 2 Legend

* denotes genes also significantly regulated in SY5Y exposed to MPP^+ alone (see Table 1 part A).

† denotes genes significantly regulated during exposure to both LY 294002 and UO 126.

“Max fold change from baseline” refers to the maximum change in signal observed for a given gene over all time points examined as compared to average (mean) signal observed in control cells.

Chapter 2 Table 3

A. Genes regulated by MPP+ and by MPP+/LY 294002 in combination

Gene	Function
ras-related protein RAB-32	Vesicular trafficking; GTPase
MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)	Intracellular signal transduction

B. Genes regulated by MPP+ and by MPP+/UO126 in combination

Gene	Function
lumican precursor (LUM); keratan sulfate proteoglycan; LDC	extracellular matrix (ECM) component
LIM domain kinase 1 (LIMK-1)	mediates Rho/actin cytoskeleton signaling via phosphorylation of cofilin
94-kDa glucose-regulated protein (GRP94)	adenotin, cell-surface 96 kDa glycoprotein; binds adenosine; similar to various stress-induced proteins
Casein kinase I alpha isoform (CKI-alpha); CK1; CSNK1A	serine/threonine protein kinase, has broad specificity
RBQ1 retinoblastoma binding protein	binds directly to Rb protein; regulates cell proliferation; interacts preferentially with underphosphorylated Rb

C. Genes regulated by LY 294002 and UO 126 in combination

Gene	Function
protein phosphatase PP2A 55-kDa regulatory subunit neuronal isoform; protein phosphatase PP2A B subunit beta; beta-PR55	Regulatory subunit of protein phosphatase 2
lysosomal acid lipase/cholesteryl ester hydrolase precursor (LAL); acid cholesteryl ester hydrolase; sterol esterase; lipase A (LIPA); cholesteryl esterase†	deacylates cholesteryl and triacylglyceryl ester core lipids
lysosome membrane protein II (LIMP II); 85-kDa lysosomal membrane sialoglycoprotein (LGP85); CD36 antigen-like 2 (CD36L2)†	significant homology with collagen type I receptor, thrombospondin receptor
insulin-like growth factor-binding protein 3 precursor (IGF-binding protein 3; IGFBP3; IBP3)†	may bind to and modulate insulin-like growth factor activity; induces early apoptosis and has potential tumor suppressive effects in prostate cancer

Chapter 2 Figure Legends

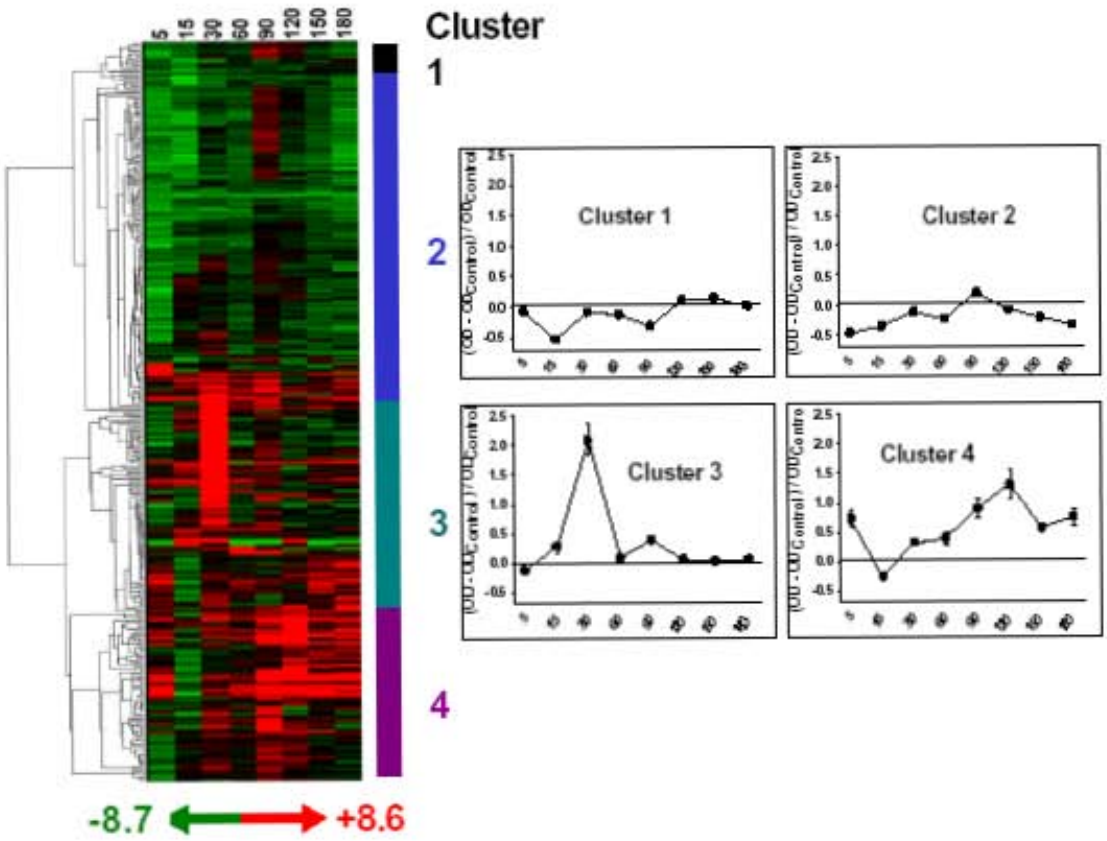
Figure 1. (Left) Gene expression changes in SY5Y cells over the time course (5-180

minutes) of MPP+ exposure, as represented by Cluster analysis (N=378 genes). Black indicates no change in expression relative to control (no MPP+); increasing intensities of green and red indicate greater depression (maximum observed=8.7 fold) and elevation (maximum observed=8.6 fold) of expression, respectively. The locations of the four mathematical clusters are indicated by the color-coded vertical bars to the right. (Right) Plots of mean expression ratios (experimental/control) for genes in each mathematical cluster at each time point of MPP+ exposure. Cluster 1, N=18 genes; cluster 2, N=171; cluster 3, N=83 genes; cluster 4, N=106 genes.

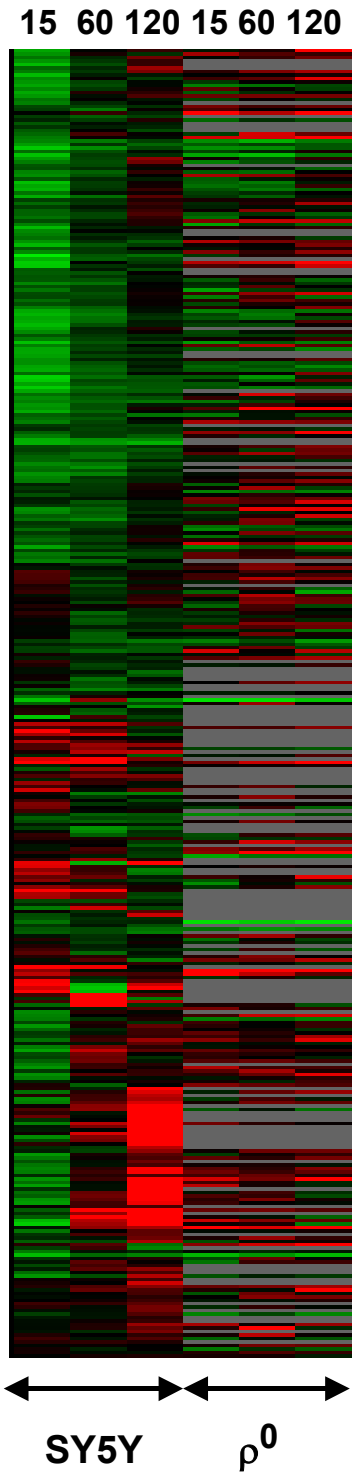
Figure 2. Gene expression changes in control SY5Y compared to mtDNA-free ρ^0 cells at selected time points of MPP+ exposure (15, 60, 120 minutes), as represented by Cluster analysis. Color code is same as for Figure 1, with the addition of gray, which indicates that the gene is not represented in the ρ^0 group.

Figure 3. (A,B) Scatterplots comparing the normalized basal gene expression levels in SY5Y control cells (x-axis) compared to expression levels in SY5Y cells pretreated with (A) U0126, an inhibitor of MEK/ERK; or (B) LY294002, an inhibitor of PI3-kinase. (C) Survival curves of SY5Y cells incubated for 24 hours

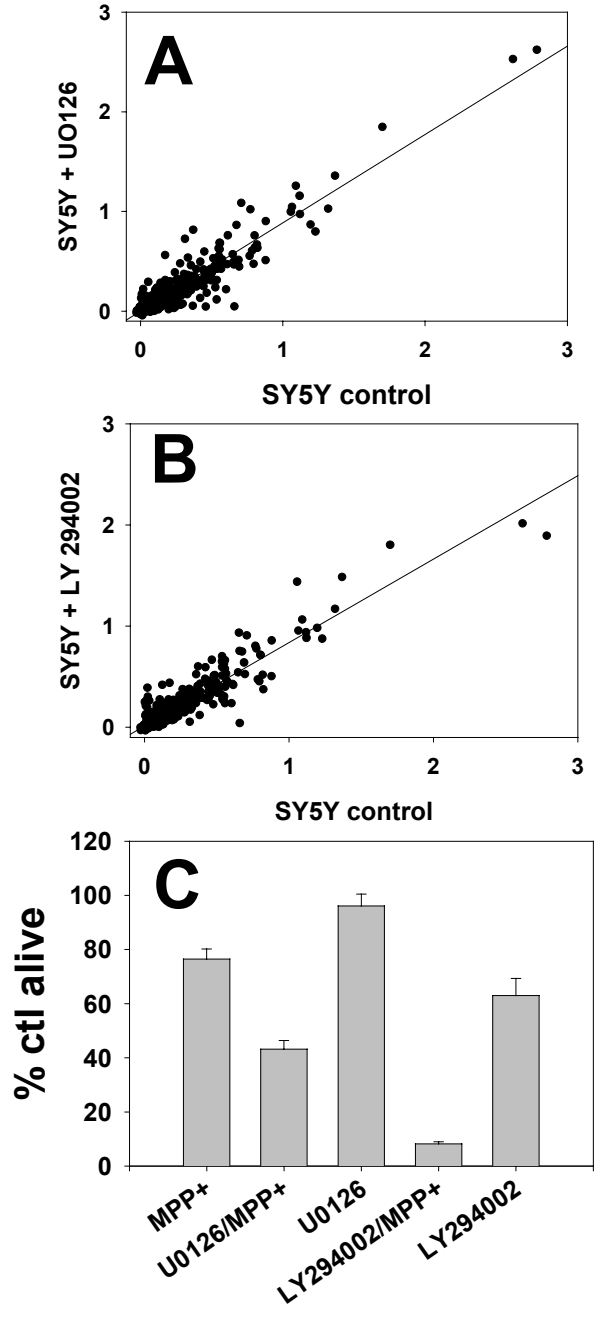
with U0126 or LY294002 either in the absence of or presence of MPP+. Data adapted from Halvorsen, et al, 2002.



Chapter 2 Figure 1.



Chapter 2 Figure 2.



Chapter 2 Figure 3.

Chapter 3

Applications of novel molecular technologies in the study of gene expression response to MPP+.

Introduction

Following our analysis of the dependence of nuclear gene expression response on mitochondrial DNA integrity, we were left with a number of intriguing potential directions for follow-up studies. The rapidity and robustness of gene expression change in response to MPP+ in our limited gene set made us very interested in how these results would or would not generalize to a larger set of genes. The profound changes in the gene expression responses that were effected by chemical modulators of cell signaling reinforced our conviction that such responses are complex, multifactorial events that would be difficult, at best to dissect through conventional molecular and biochemical techniques. As compared to our nylon array study, we observed that larger numbers of genes were altered in their expression as a result of MPP+ exposure; this is not surprising considering the expansion of the number of genes assayed per array. Given the dependence in MPP+-exposed cells of molecular events and cell signaling upon both nitric oxide and NFkB activation that has been demonstrated by previous work in our group, we tested the hypothesis that transcriptosome changes following MPP+ exposure might have similar dependencies. We were intrigued to find that ~90% of a large number of genes that were regulated by MPP+ appeared to be dependent on NO or NFkB translocation to the nucleus.

Not only were these findings consistent with previous observations, but they suggested that mitochondria might use endogenously produced NO as a signaling molecule to communicate ETC impairment to the nuclear transcriptosomal machinery, and that NFkB transcription factor played a significant upstream regulatory role in this process. We also found that the magnitude of gene response was much greater at the 15m timepoint than that at 90m of MPP+/drug exposure. This datum reinforces the notion, first articulated following our nylon array work, that gene expression changes in response to MPP+ are very rapid, and can change relatively quickly (or at least more quickly than a process that might rely on turnover and synthesis of proteins *de novo*). One potential weakness of our protocol is that we continue to make use of the MPP+ SY5Y model; as noted previously the acute nature of cell death in this model does not follow a similar timecourse as the prolonged attrition of cells that occurs in the PD process. While acknowledging this fact, the ease of availability and reproducibility that we have observed with RNA samples from SY5Ys made them the best available choice for this initial study of the relationship between acute mitochondrial bioenergetic impairment and changes in the cell transcriptosome.

While a comprehensive evaluation of every gene expression level measured is clearly beyond the scope of our work, we discuss several genes identified in the microarray screens that may play as yet unidentified roles in MPP+ neurotoxicity. Finally, we describe our efforts to alter the course of MPP+ induced cell death through several RNA interference methods, in addition to

presenting a preliminary analysis of the ontological categorizations of transcriptionally regulated genes in our experimental system using a publicly available software package, GoMiner.

Materials and Methods

Cell Culture and Treatment.

SH-SY5Y cells were obtained from the American Tissue Culture Collection (ATCC; Manassas, VA) and maintained in culture as previously described (Cassarino et al., 1997; Cassarino et al., 2000; Swerdlow et al., 1996). 3×10^6 SY5Y cells were placed onto 10 cm tissue culture plates (Greiner Bio-One; Longwood, FL) and maintained at 37°C for 24 hours prior to exposure to 5 mM MPP^+ (Sigma-Aldrich; St. Louis, MO) for 15m or 90m in the presence or absence of 15m of pretreatment with either the nitric oxide scavenger 2-Phenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl (PTIO) (Akaike et al, 1993) at 1 mM or the nuclear factor kappa B nuclear translocation inhibitor peptide SN50 (Lin et al, 1995) at 18 μM (both obtained from Calbiochem). Cultures were also exposed to carrier (phosphate buffered saline) alone, and to SN50 or PTIO in the absence of MPP+.

RNA Isolation.

Total RNA was isolated from cell cultures immediately following completion of any MPP^+/SN50/PTIO exposure using the RNEasy Mini system

(Qiagen; Valencia, CA), according to manufacturer's protocol. RNA samples were archived at -80°C.

cDNA Arrays.

The general experimental plan for our microarray protocol is diagrammed in Figure 1. cDNA probes were prepared from each total RNA sample and hybridized to "human 19k3" cDNA microarrays (University of Toronto Microarray Centre, Toronto, Ontario) using the Perkin Elmer MicroMax kit according to manufacturer's protocol. Briefly, each RNA isolate is reverse transcribed into cDNA that incorporates either fluorescein or biotin moieties on cytosine residues via provision of deoxycytosine so labeled. In our experiments, cDNA from control SY5Y samples always bears fluorescein, and experimental samples are labeled with biotin. These labeled cDNA populations are mixed and hybridized to the microarrays overnight under constant temperature and humidity. Following a series of washes, the arrays are exposed to an anti-fluorescein antibody-horseradish peroxidase conjugate. This localizes HRP to the regions of fluorescein-cDNA hybridization. Application of a Cyanine 3-conjugated tyramide compound catalyzes the rapid deposition Cyanine 3 tyramide immediately adjacent to the immobilized HRP. In this manner, many fluorescent molecules are deposited at the site of hybridization, resulting in signal amplification relative to using cDNAs that are synthesized to directly incorporate fluorescent moieties. Following Cy3 deposition, residual HRP is inactivated and the array is exposed to streptavidin HRP, which binds to the biotinylated nucleotides. In a similar

reaction to the Cy3 process described, the conjugated HRP activity is used to catalyze deposition of Cy5 tyramide. In this way, the signals from the 2 RNA samples are amplified with separate fluorophores on the same array. Two hybridizations were carried out in which both Cy3 and Cy5 fluorophores were associated with control (untreated) SY5Y cDNA to determine the prevalence of fluorophore specific alterations ("dye-swap" effects) on gene expression signals. Array hybridization fluorescence digital images were acquired and saved in .TIF format using ScanArray software package with a ScanArray 4000 microarray scanner (Packard BioChip Technologies).

Statistical Analysis.

A major limitation of many published microarray studies is limited biological and technical replication, with complex statistical approaches used to extract significance in the absence of replication. Our approach to avoid this limitation was to perform for each experimental condition three independent biological replications (cell culture, drug exposure, RNA isolation) and duplicate hybridizations for each independent biological sample. Each hybridization experiment comprised the hybridization of cDNA samples derived from experimental and control (untreated) SY5Y cells, labeled with cyanine 5 or cyanine 3 fluors respectively, to cDNA microarray slides. Fluorescence intensity and background levels for each gene hybridization were extracted from TIFF files using the QuantArray software package. Net hybridization intensities for individual genes were obtained through background subtraction followed by

normalization to the mean hybridization signals for each individual microarray *in toto*, allowing direct comparisons across experimental conditions. Following normalization, gene hybridization intensities greater than 2x the signal obtained from a standardized region of the array with no adsorbed DNA were used in downstream analyses. For experiments in which SY5Y cells were treated with MPP^+, PTIO or SN50 alone, genes that exhibited a $\pm$ 2-fold change were considered to have altered expression. For experiments involving comparison of MPP^+ alone to MPP^+ in combination with PTIO or SN50, Student's t-tests were used to compare ratios of hybridization intensities of experimental and control samples for each individual gene spot in each treatment condition. P values were calculated for each gene, ranked from least to greatest and compared to their corresponding critical p values obtained with the Benjamini-Hochberg calculation(p_{BH}) with significance set at $p \leq 0.05$. P values less than P_{BH} were considered significant.

The control-control hybridizations to check for dye-swap effects were processed in the same way as described for the other hybridization experiments above, and array-normalized, background subtracted intensity ratios Cy5:Cy3 were compared. The results are presented in Figure 2.

Gene Ontology Analysis

We attempted to define biological meaning to the transcript changes by looking for correlations among gene ontology groups in our array datasets with "GoMiner" (Zeeberg, et al, 2003; http://genomebiology/2003/4/4/R28), an open-

source program that organizes gene lists in the context of Gene Ontology categories (Ashburner, et al, 2000). GENBANK ID's of our microarray clones were annotated to gene symbols using DAVID (Database for Annotation, Visualization and Integrated Discovery; Dennis, et al, 2003; http://genomebiology/2003/4/9/R60). The gene symbols from DAVID were then annotated with a (+1) or (-1) to indicate increased or decreased expression, respectively, and analyzed with the GoMiner engine. The output from GoMiner includes both grouping of experimental genes into Gene Ontology groups and a "relative enrichment" (RE) calculation for each Gene Ontology group (RE={(changed genes in category/total genes in category)/(changed genes in chip/total genes in chip)}). We used the GoMiner RE values to test the hypothesis that two different experimental manipulations altered Gene Ontology groups in similar ways by constructing correlation plots of the RE values for genes increased or decreased in expression in the different experimental conditions. We used RE values for the second order nodes in the GoMiner output for the initial correlations. For second order nodes where n>1000 genes, we also analyzed correlations for the third order nodes in the GoMiner output.

RT-PCR.

cDNA was prepared from experimental total RNA samples using the Advantage RT-for-PCR kit (Clontech Laboratories) using oligo(dT)primers according to the manufacturers directions. PCR was carried out using the Advantage cDNA PCR kit (Clontech Laboratories), with the indicated PCR

primers according to manufacturer's directions. Samples were held at 94^{o}C for 1 minute, followed by 33 cycles of [94^{o}C-30 seconds, 68^{o}C-2 minutes], followed in turn by a final 68^{o}C for 5 minutes. PCR products were separated on 2% agarose gels and visualized by ethidium bromide incorporation. SYN2 and GA3PDH were selected for RT-PCR verification of both statistically significant and non-significant gene expression changes.

SN50 effects on MPP+ induced cell death.

Cultures of 10^4 SY5Y cells were plated in individual wells of Corning 96 well clear bottom, black plates with DMEM and 10% FBS. Cells were treated with either MPP+ (5 mM), SN50 peptide (18 μM) or a combination of the two for time periods ranging from 2 to 24 h. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-based spectrophotometric assay. The results are depicted in Figure 3. Data compiled by Dr. Jameel Dennis indicate that PTIO at the concentrations used in the present experiments has no measurable effect on MPP+ induced cell death through 24h of concurrent exposure (Dennis and Bennett, 2003).

RNA interference.

As described in chapter 1, RNA interference refers to the catalyzed destruction of specific RNA sequences via the introduction of short double stranded RNA species of approximately 10-20 base pairs, so-called short interfering RNAs (siRNAs). The general sequence of events in this process is

depicted in Figure 4. This technology was appealing to us as it presented a seemingly direct route to modulation of the gene expression signals we observed in our microarray work, and evaluation of their effects on cell death in our model system. Several methods were used to generate siRNA directed against SYN2, DDIT3, FGD1 and RET gene products. Methods employed included chemical synthesis of siRNA, *in vitro* transcription of complementary dsRNAs that were then subjected to RNAse III digestion to produce siRNAs, and transfection of SY5Y cells with vectors designed to drive constitutive expression of siRNA hairpin species under the control of RNA polymerase II.

To date, we have had siRNAs chemically synthesized for SYN2, DDIT3 and FGD1, though none have proven effective in decreasing their respective RNA signals as measured by RT-PCR assay. Figure 5 depicts typical RT-PCR results following transfection of SY5Y cells with our chemically synthesized siRNAs. In part A, lane L is a 100 bp DNA ladder, lane 1 is a positive control PCR product, lanes 2 and 3 are FGD1 RT-PCR products from SY5Y cells transfected with control and FGD1 siRNA, respectively, and lanes 4 and 5 are RT-PCR products from SY5Y cels transfected with control and DDIT3 siRNA, respectively. In part B of Figure 5, lane 1 is a positive control PCR product, while lanes 2 and 3 contain SYN2 PCR products from SY5Y cells transfected with control and SYN2-directed siRNAs, respectively. Lanes 4 and 5 and lanes 6 and 7 duplicate lanes 2 and 3. The lack of attenuation of signal in any of the gene product-directed siRNA transfected samples suggests that the siRNAs are ineffective at silencing their targets.

For *in vitro* transcription, T7 promoters were appended to FGD1 and SYN2 PCR products and used to generate an approximately 200 bp double stranded RNA corresponding to a C-terminal region of the transcript, which was subjected to RNAse III digestion. The products of this digestion were transfected into SY5Y cells, and they were not found to be efficacious in lowering FGD1 expression by RT-PCR; in Figure 6, Lane "L" contains a 100 bp DNA ladder, lane 1 is a positive control PCR product, lanes 2 and 3 are SYN2 PCR products from SY5Y cells transfected with control siRNA or FGD1 siRNA respectively, and lanes 4 and 5 duplicate lanes 2 and 3. The presence of PCR products in both control and directed siRNA transfected samples suggests that the silencing protocol is ineffective.

Vectors encoding hairpin siRNAs directed against C-terminal regions of SYN2, RET, FGD1 and DDIT3 gene products were cloned into *pSilencer* expression vectors (Ambion), and transfected into SY5Y cells, but unexpectedly high cellular toxicity was observed following hygromycin selection of transfected cells, despite high estimates of transfection efficiency and multiple successful transfection experiments utilizing the same cell line with different plasmid expression vectors in the past. No positive transformants were recovered following hygromycin selection with any of the *pSilencer* constructs. A diagram of the *pSilencer* vector used is presented in Figure 7.

Results

cDNA Microarrays

Following background subtraction, normalization, log transformation and comparison to control RNA hybridization, a total of 533 genes of 19,200 total (2.7%) were found to have increased or decreased 2-fold in response to 15 minutes of 5 mM MPP+ exposure. These genes are listed, along with available LocusLink identifers and Gene Ontology annotations, if available, in Appendix A. Of these, were 409 were upregulated and 124 were downregulated. Of these 533, 65 (11.7%) were found to be significantly altered in their expression in the presence of PTIO, and 93 (16.7%) were found to be significantly altered in their expression in the presence of SN50 peptide. These genes are listed, along with available LocusLink and Gene Ontology information in Appendices B and C. The relative sizes of these gene groups are presented in Figure 8, part A.

At 90 minutes of MPP+ treatment, 143 genes (0.7%) were found to have increased or decreased 2-fold or more as compared to control. These genes and available annotations for them are listed in Appendix D. Of these 143 genes, 136 were upregulated and 7 were downregulated. Of these 143, 2 (cellular retinoic acid-binding protein 2:CRABP2 and integrin alpha 1:ITGA1) were found to be significantly altered in their expression in the presence of PTIO with MPP+, and none changed significantly in the presence of SN50 peptide with MPP+.

Forty genes were found to have changed +/- 2 at both 15m and 90m MPP+ timepoints. These genes and available annotations are listed in Appendix

E. The relative representation of these genes in relation to the total number of genes assayed by cDNA microarray is presented in Figure 8, part B.

A gene by gene comparison of between the 15m MPP+ and 15m MPP+ with PTIO conditions revealed 706 genes differentially regulated. This response, while similar in magnitude to that observed in the comparison of 15m MPP+ to control in numbers of genes, comprises an almost completely distinct set of genes (65/706, or greater than 90% dissimilar). These genes and any available annotative information are listed in Appendix F. When a similar comparison between the 15m MPP+ and 15m MPP+ with SN50 condition is carried out, 492 genes are found to be differentially regulated. These genes are listed in Appendix G. At 90m of MPP+ treatment, the presence of PTIO induces differential expression of 74 genes listed in Appendix H. Only one gene, ADAR3, is differentially expressed at 90m of MPP+ treatment in the presence of SN50 peptide. 73 genes were noted to be significantly changed between the 15m MPP+ condition and 15m MPP+ with either PTIO or SN50. These genes and any available annotative information are presented in Appendix I.

Gene Ontology Correlations

The correlation plots for GoMiner RE values revealed limited correlations between Gene Ontology families in the experiments where NO was scavenged with PTIO during MPP+ exposure (Figure 9) or NFkappaB translocation to the nucleus was blocked by SN50 peptide during MPP+ exposure (Figure 10). The limited correlations were more prominent in the plots of third order node RE

values and were seen for genes both downregulated (green) and upregulated (red). These Gene Ontology findings are consistent with the analysis of transcriptosome responses on a gene-by-gene basis in these experiments, in which the majority of genes altered by MPP+ alone were not altered when PTIO or SN50 were included with the MPP+.

We then compared the Gene Ontology families that were sensitive to the presence of the NfkappaB antagonist SN50 to those that were sensitive to the presence of the NO scavenger PTIO when SH-SY5Y cells were exposed to MPP+. In contrast to the previously described limited RE correlations between transcriptosome responses of PTIO+MPP+ or SN50+MPP+ with those of MPP+ alone, we observed very strong correlations between the RE values of SN50-sensitive genes and PTIO-sensitive genes (Figure 11).

Discussion

As in our previous nylon-based array experiments, we noted a rapid and robust nuclear gene expression response to the application of the mitochondrial neurotoxin MPP+ to SH-SY5Y cells. Upon examination of our data, it becomes apparent that there are major differences in gene expression during the time course of MPP+ exposure. The magnitude of expression changes is considerably higher at 15m than at 90m, which is in accord with a key finding of our nylon array work, that expression change is greatest early in the timecourse of MPP+ exposure. The fact that only 40 genes are changing +/- 2 fold at both

15m and 90m would argue strongly for the presence of distinct “early” and “late” effects of MPP+ on gene expression. Functional categorizations available for genes that change at the 15m timepoint are presented in Table 1 and those for the 90m timepoint in Table 2. It is evident that the numbers of genes listed in each of these tables is considerably smaller than the total number of genes identified to change +/- 2 fold for each condition; this illustrates a major issue confronting those working with all types of genomic datasets, that of curation. The ontological categorizations that are attached to a given gene ID are subjective; that is to say, they are susceptible to errors of classification and omission due to the fact that they must be assigned by one or at most a small group of persons acting together, relying in large part only on publicly accessible data of widely varying quality. This makes it untenable, in the majority of cases of microarray studies of mammalian samples in particular, to derive large scale conclusions about interconnections of various metabolic and cell signaling pathways in the absence of a higher order (e.g., ontological) analysis. With this limitation in mind, we will note that the largest ontological categories represented at the 15m timepoint, when the majority of changes appear to occur, are DNA binding and modifying enzymes, and protein kinases. This is in accordance with our earlier finding that the majority of early gene expression changes induced in the SY5Y cells by MPP+ are related to intracellular signalling. We also note that the finding of such rapid gene expression response is consistent with rapid mitochondrial-nuclear communication, as no experimental evidence has as yet demonstrated MPP+ to exert direct effects on nuclear gene expression, and as

we have previously demonstrated that the nuclear gene expression response induced by MPP+ is dependent on mitochondrial status.

The most common gene ontology categories represented in the genes that are differentially expressed at 15m of MPP+ treatment in the presence of PTIO are listed in Table 3, and those of genes that vary significantly in the presence of SN50 peptide at 15m of concurrent MPP+ treatment are listed in Table 4. The most common categorizations in both cases are gene products that bind nucleic acids, and protein kinases, consistent with an intracellular signalling response regulating transcription of different genes.

Perhaps one of the most interesting findings in this dataset are the startling effects seen with PTIO and SN50 treatment concurrent with 15m of MPP+ exposure. The number of genes differentially expressed in the presence of PTIO is roughly equivalent to the number that vary +/- 2 fold or greater with 15m MPP+ exposure as compared to control, yet the groups of genes are almost totally dissimilar. A similar effect is seen with SN50, with about 80% dissimilarity noted. These results strongly imply that the nuclear gene expression response induced by this mitochondrial neurotoxin is dependent upon nitric oxide and/or NFkB signaling. The finding that the vast majority of gene expression changes documented at both 15m and 90m of MPP+ exposure do not occur in the presence of inhibitors of NFkB nuclear translocation or of a nitric oxide scavenging compound suggests that these expression responses are mediated through NFkB and/or nitric oxide. As mitochondria possess intraorganellar nitric oxide synthase, these data are consistent with nitric oxide being responsible for

signal transduction to the nucleus. NFkB's role in translocation to the nucleus and upregulation of gene transcription there is well known, and our data are consistent with it being involved in the mitochondrial-nuclear signaling that we have observed.

One important aspect of high-density microarray studies that has been neglected by many researchers is the issue of multiple hypothesis testing. When parametric statistical analyses are applied to datasets involving thousands of individual comparisons, the chance of introduction of type I (false positive) errors grows dramatically (for an excellent review, see Westfall and Young, 1993). A familiar method of compensating for this effect is the Bonferroni correction, in which the p value associated with a significant result for a given test (α) is altered by the formula α / n, where n is the number of simultaneous tests. For example, if t tests were used to evaluate microarray results for significance at α = 0.05, and the array comprised a total of 1000 gene spots, the p value associated with statistical significance would be 0.05 / 1000, or 5 x 10^{-5}. This transformation, while undeniably effective at reducing type I errrors, is generally considered to sacrifice too much statistical power in the process. Indeed, when the Bonferroni correction is applied to the t statistics that we calculated to compare gene expression changes with MPP+ in the presence of PTIO or SN50, a p value of 2.6 x 10-6 is required to achieve significance (α of 0.05 divided by 19,200 gene features/array). With regards to our microarray datasets, the Benjamini-Hochberg transformation, while among the least restrictive of the multiple testing

correction algorithms available, strikes a reasonable balance between elimination of false positives and inclusiveness of potentially "real" differences in gene expression based on the number of genes that meet significance criteria. This method entails the calculation of p values followed by their ranking from lowest to highest, 1 through n. For type I error control at level α, a critical p value is calculated for each ranked position in the list, by mulitplying the rank by the desired α, and dividing by n, the total number of rankings. Initial p values for each test are then compared to the p critical value for the test's ranking. P values less than or equal to p critical are considered to be significant, with the chance of false discovery error less than or equal to α. For our experiments, α is always set equal to 0.05.

Several lab groups have recently described methods using Bayesian networks for the statistical analysis of microarray data. These methods have the advantage of being able to assign unambiguous confidence intervals for differences between unlimited numbers of sets of gene expression data, and are particularly useful when the number of samples is low and/or some expression values are missing, but suffer from the same potential for false-discovery errors as more conventional parametric analyses do when making thousands of comparisons simultaneously. We have preliminarily evaluated one publicly available software package (Bayesian Analysis of Gene Expression Levels, BAGEL, Townsend and Hartl, 2002) which performs such analyses, and at this

time, in light of the quality of our data sets, do not feel that it offers us significant improvements over the parametric methods that we have presented here.

Multiple laboratories have reported significant “dye-swap” effects, in which gene expression levels recorded for particular genes in microarray analyses vary depending on the fluorophore associated with the cDNA hybridized to the array. The tyramide-signal amplification protocol that we employed avoids the majority of these issues, as the fluorophore deposition is in great excess to the number of labeled nucleotides in an individual hybridized cDNA, but it was heartening to note that these effects only occur in a very small number of cases, less than 5% of all gene spots, as shown by our Figure 2 findings. From this, we can move forward with future array experiments with the tyramide system knowing that dye-swapping is not necessary in this context, saving time and expense.

It is clear from our data that major changes in regulation occur between the time points that we are studying. We began to study the generated lists for genes that displayed interesting regulatory characteristics, and that also might have relevance to neurodegenerative processes in general and PD in particular.

The first such gene that we have identified is synapsin 2 (SYN2). SYN2 is a member of the synapsin family of genes which encode neuronal phosphoproteins associated with the cytoplasmic surface of synaptic vesicles. Family members are implicated in synaptogenesis and the modulation of neurotransmitter release, suggesting a potential role in several psychiatric and neurodegenerative diseases. This member of the synapsin family encodes a neuron-specific phosphoprotein that selectively binds to small synaptic vesicles

in the presynaptic nerve terminal. SYN2 is upregulated approximately 4 fold in response to 15m of MPP+ treatment.

The second gene that we have singled out for downstream analysis is faciogenital dysplasia 1 (FGD1). FGD1 can bind specifically to the Rho family GTPase Cdc42Hs and stimulate the GDP-GTP exchange of the isoprenylated form of Cdc42Hs. It also stimulates the mitogen activated protein kinase cascade leading to c-Jun kinase SAPK/JNK1 activation. FGD1 has an essential role in embryonic development, and FGD1 gene mutations result in the human developmental disorder, Aarskog-Scott syndrome. We were especially interested in FGD1 in light of the key roles played by the MAP kinase cascade in MPP+ signalling described by previous work in the CSND. FGD1 is upregulated approximately 3 fold in response to 15m of MPP+ treatment.

The third gene to be considered is DNA-damage-inducible transcript 3 (DDIT3). It is a nuclear transcription factor shown to be upregulated in a variety of situations where genomic damage is induced by oxidative stress and other means. This makes DDIT3 a potential transducer of oxidative damage signals from mitochondria to the nucleus, a process that we have shown is dependent on mitochondrial DNA status. DDIT3 is downregulated to approximately 33% of control levels in response to 15m of MPP+ treatment.

The final gene that we identified is the ret proto-oncogene (RET). This gene is a member of the cadherin superfamily, and encodes a receptor tyrosine kinase, which are cell-surface molecules that generally transduce signals for cell growth and differentiation. RET plays a crucial role in neural crest development,

and it can undergo oncogenic rearrangement both *in vivo* and *in vitro*. Mutations in this gene are associated with the disorders multiple endocrine neoplasia, type IIA, multiple endocrine neoplasia, type IIB, Hirschsprung disease, and medullary thyroid carcinoma. Alternative splicing has been described, with at least 4 transcript variants. Most importantly for PD considerations, RET comprises one half of the cell surface receptor complex for glial-derived neurotrophic factor (GDNF), which was identified in 1993 as a key survival factor for dopaminergic neurons in the striatum. Several clinical trials are underway in which GDNF is infused into the putamens of PD patients in hopes of slowing the neuronal cell death processes ongoing. If mitochondrial neurotoxicity is a key component of the apoptotic process, RET may be one of the molecules that is under its regulation. In our dataset, RET is found to be decreased to approximately 29% of control expression after 15m of MPP+ treatment.

The fact that we have encountered great difficulties in implementing our RNAi strategy is disheartening, but we still feel that it provides the most logical method for directly and specifically mediating gene expression in a wide variety of experimental systems, and hope to pursue it in the future in this and other disease models. Initial publications were highly enthusiastic regarding the ease of use and efficacy of these technologies, but subsequent experience in our laboratory and others, is proving contrary.

Finally, our intial experiences with the GoMiner package have proven extremely valuable. The fact that groups of genes may be highly functionally correlated, while being highly dissimilar at the individual gene identity level,

encourages us to pursue this type of analysis further. We believe that directed analysis of curated gene families provides the best possible system to date for attempting to assign biological significance to the growing amount of publicly available microarray data. With regards to our data, the major finding made possible by GoMiner, that both nitric oxide and NFkB seem to regulate the same biological response to MPP+ in SY5Y cells represents a novel investigative tack that may well prove valuable in the design of future experiments.

Chapter 3 Table 1. Ontological categorization of genes that change +/- 2 fold after 15m MPP+ exposure.

CATEGORY	NUMBER
purine nucleotide binding	36
DNA binding	29
hydrolase activity, acting on acid anhydrides	16
transferase activity, transferring phosphorus-containing groups	16
protein kinase activity	14
hydrolase activity, acting on ester bonds	13
RNA binding	11
peptidase activity	11
transmembrane receptor activity	11
calcium ion binding	10
cytoskeletal protein binding	10
transition metal ion binding	9

Chapter 3 Table 2. Ontological categorization of genes that change +/- 2 fold after 90m MPP+ exposure.

CATEGORY	NUMBER
DNA binding	9
calcium ion binding	5
purine nucleotide binding	5
transition metal ion binding	4
hydrolase activity, acting on acid anhydrides	3
hydrolase activity, acting on ester bonds	3
protein kinase activity	3
transferase activity, transferring phosphorus-containing groups	3
lamin binding	2
magnesium ion binding	2
peptidase activity	2

Chapter 3 Table 3. Ontological categorization of genes significantly different between 15m MPP+ exposure and 15m MPP+ with PTIO.

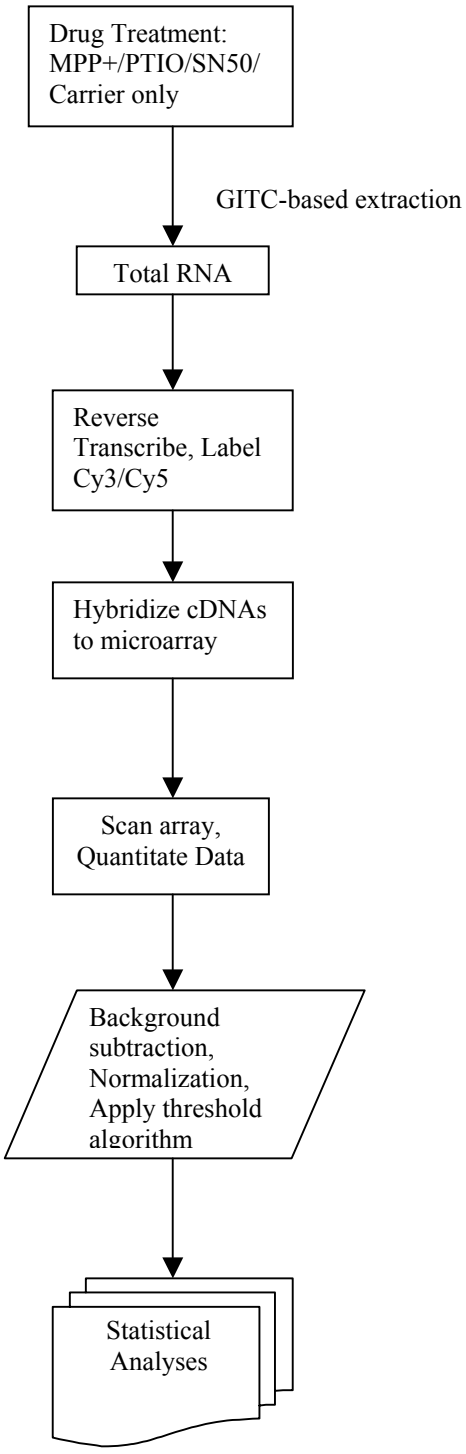
CATEGORY	NUMBER
nucleotide binding	32
DNA binding	26
transferase activity, transferring phosphorus-containing groups	22
protein kinase activity	18
hydrolase activity, acting on acid anhydrides	14
RNA binding	13
peptidase activity	12
transition metal ion binding	12
calcium ion binding	9
hydrolase activity, acting on ester bonds	9
primary active transporter activity	7
small GTPase regulatory/interacting protein activity	7
transmembrane receptor activity	7
cytoskeletal protein binding	6
alpha-type channel activity	5
cytokine activity	5
transcription factor binding	5
Unclassified	571

Chapter 3 Table 4. Ontological categorization of genes significantly different between 15m MPP+ exposure and 15m MPP+ with SN50

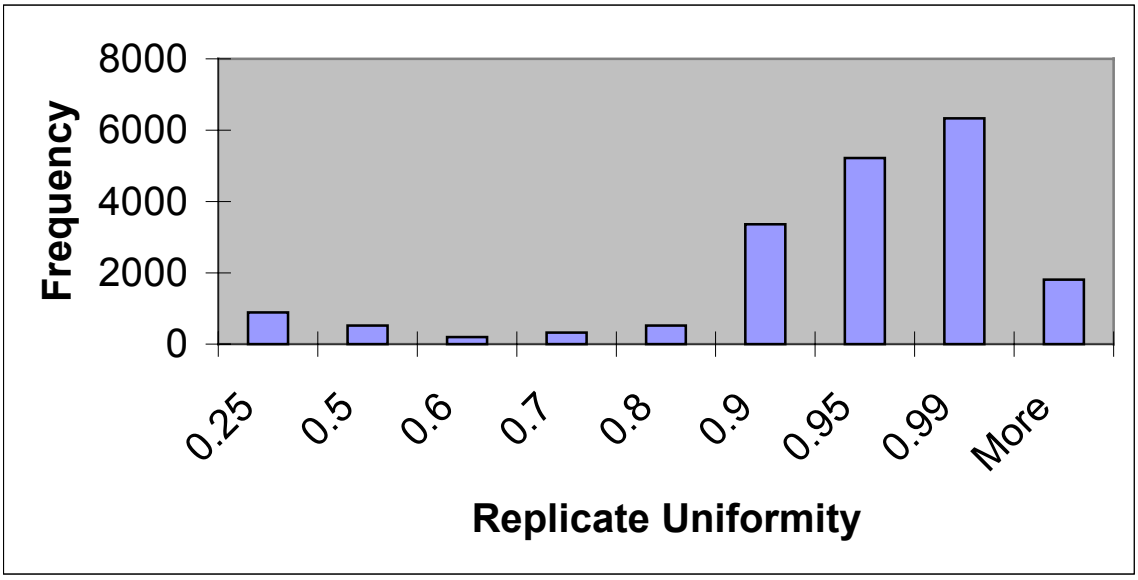
CATEGORY	NUMBER
purine nucleotide binding	18
DNA binding	16
transferase activity, transferring phosphorus-containing groups	15
calcium ion binding	13
protein kinase activity	11
RNA binding	10
cation transporter activity	9
transition metal ion binding	9
transmembrane receptor activity	9
hydrolase activity, acting on acid anhydrides	8
peptidase activity	8
hydrolase activity, acting on ester bonds	7
primary active transporter activity	6
unclassified	399

.

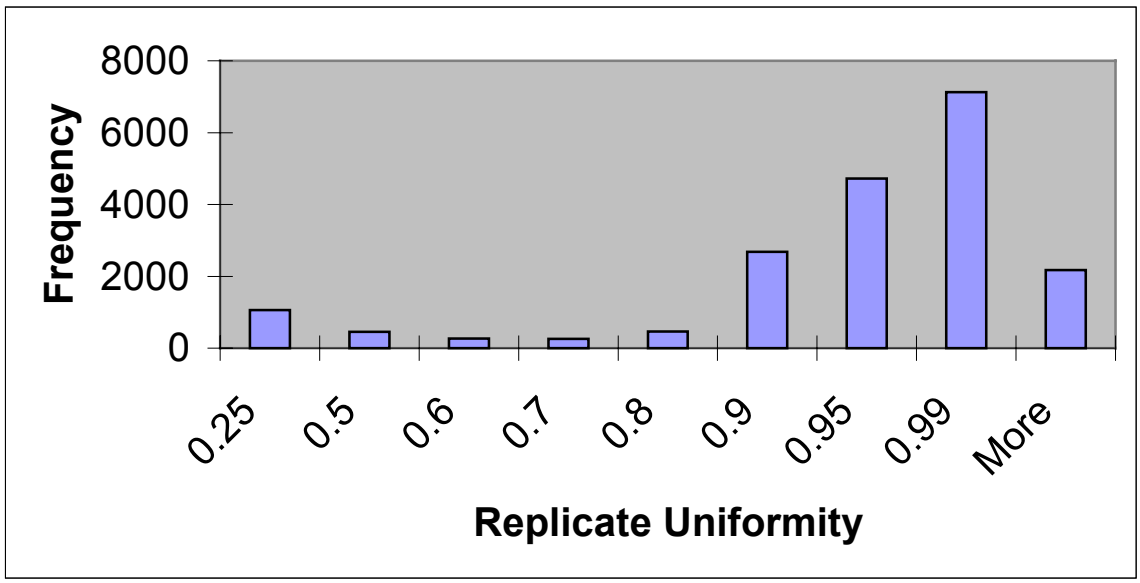
Chapter 3 Figure 1. Outline of generalized microarray experimental procedure.



Chapter 3 Figure 2. Analysis of “dye-swap” microarray replicates.

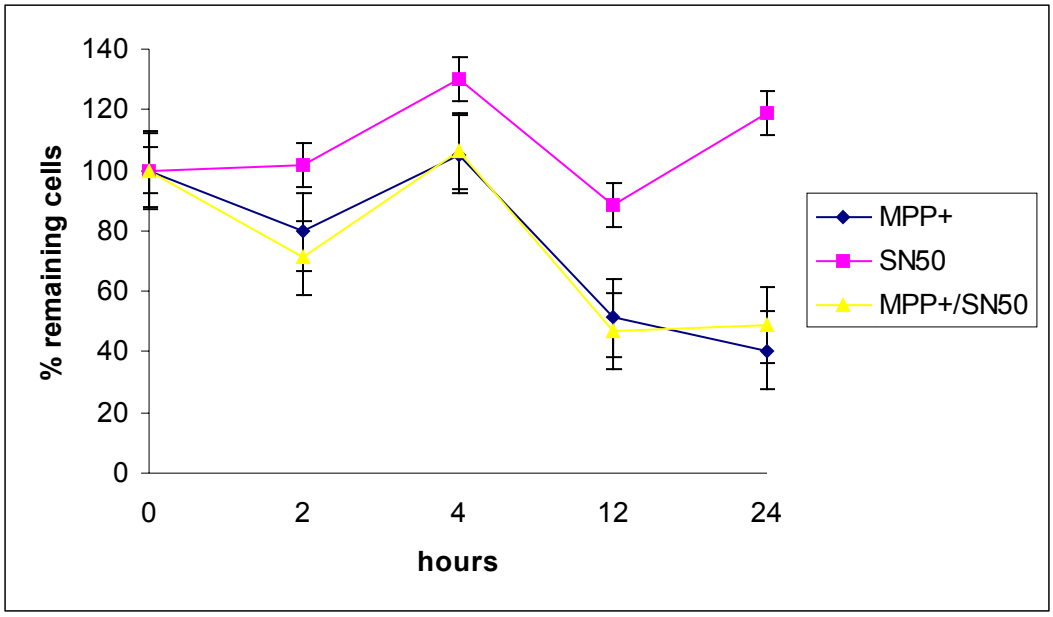


Hybridization 1: Histogram of Gene-Spot Cy5:Cy3 Uniformity Frequencies

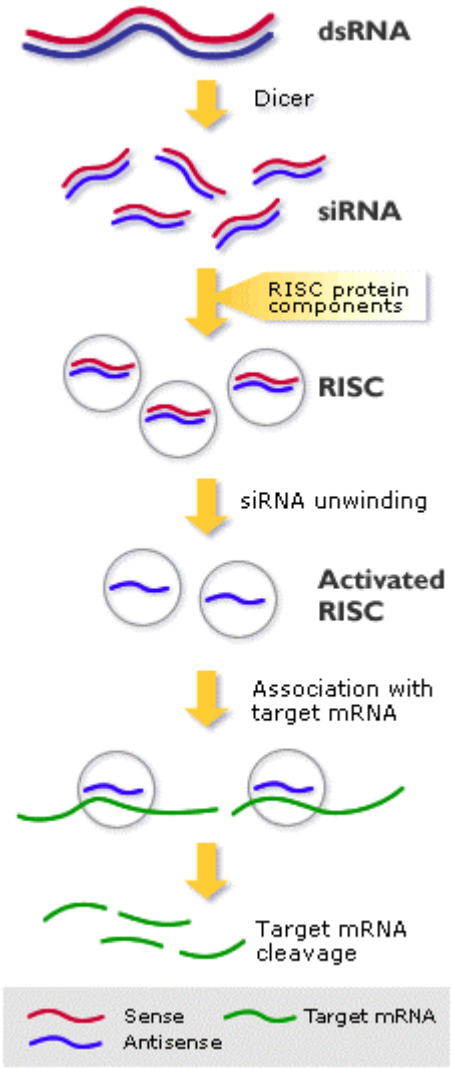


Hybridization 2: Histogram of Gene-Spot Cy5:Cy3 Uniformity Frequencies

Chapter 3 Figure 3. SN50 peptide does not affect MPP+ induced cell death in SY5Y neuroblastoma.

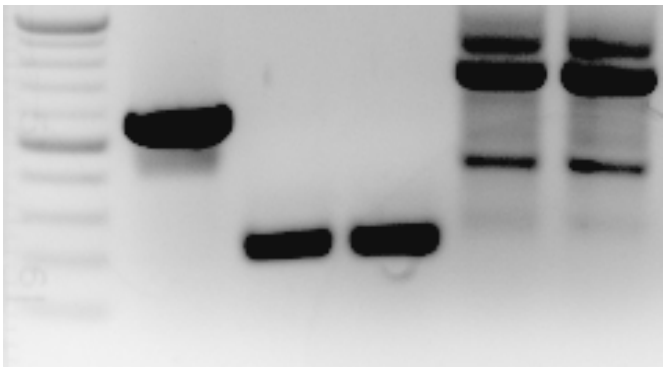


Chapter 3 Figure 4. General Mechanism of RNA Interference via dsRNA.



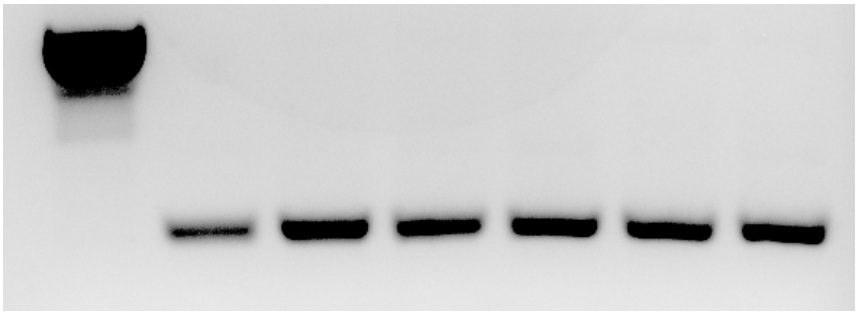
Chapter 3 Figure 5. Transfection of Chemically Synthesized siRNAs.

A. FGD1, DDIT3



Assay for chemically synthesized siRNA efficacy: (L) 100 bp DNA ladder. (1) positive control PCR product. (2-3) FGD1 PCR products indicating no silencing. (4-5) DDIT3 PCR products indicating no silencing. See Methods for details.

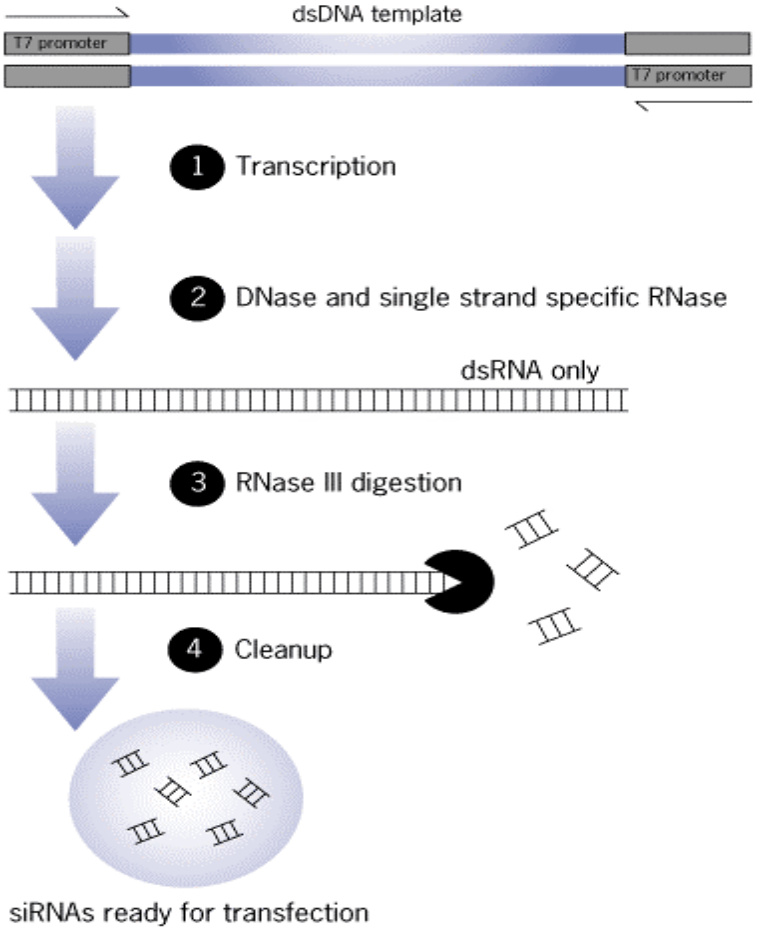
B. SYN2



Assay for chemically synthesized siRNA efficacy: (1) positive control PCR product. (2-3) SYN2 PCR products indicating no silencing in SYN2 and control siRNA transfected cells, respectively. (4-5, 6-7) duplicates of lanes 2 and 3. See Methods for details.

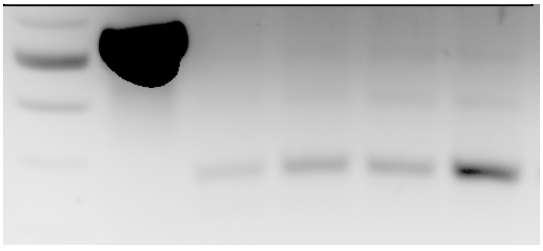
Chapter 3 Figure 6

A. Experimental design for *In vitro* transcription/RNAse III siRNA production for use in mammalian gene silencing.



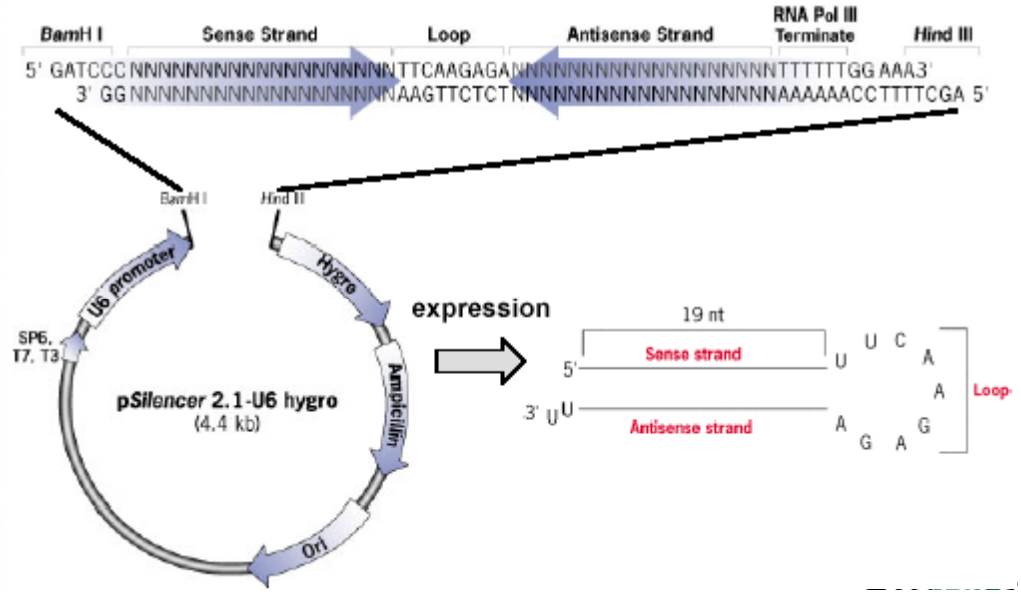
Artwork ©Ambion, Inc.

B.



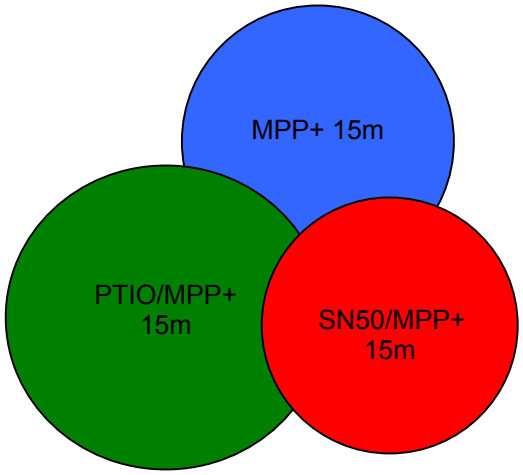
PCR Assay for efficacy of *in vitro* transcribed siRNA. (L) 100 bp DNA ladder. (1) Positive control PCR. (2,4) SYN2 PCR products indicating no silencing in siRNA transfected cells. (3,5) SYN2 PCR products in control siRNA transfected cells.

Chapter 3 Figure 7. Example of a plasmid expression vector for siRNA induction in mammalian cells.

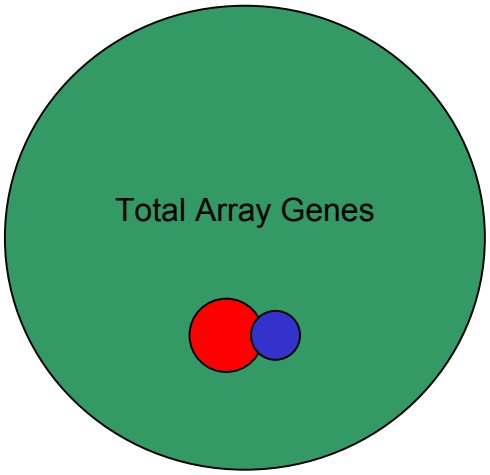


Artwork ©Ambion, Inc.

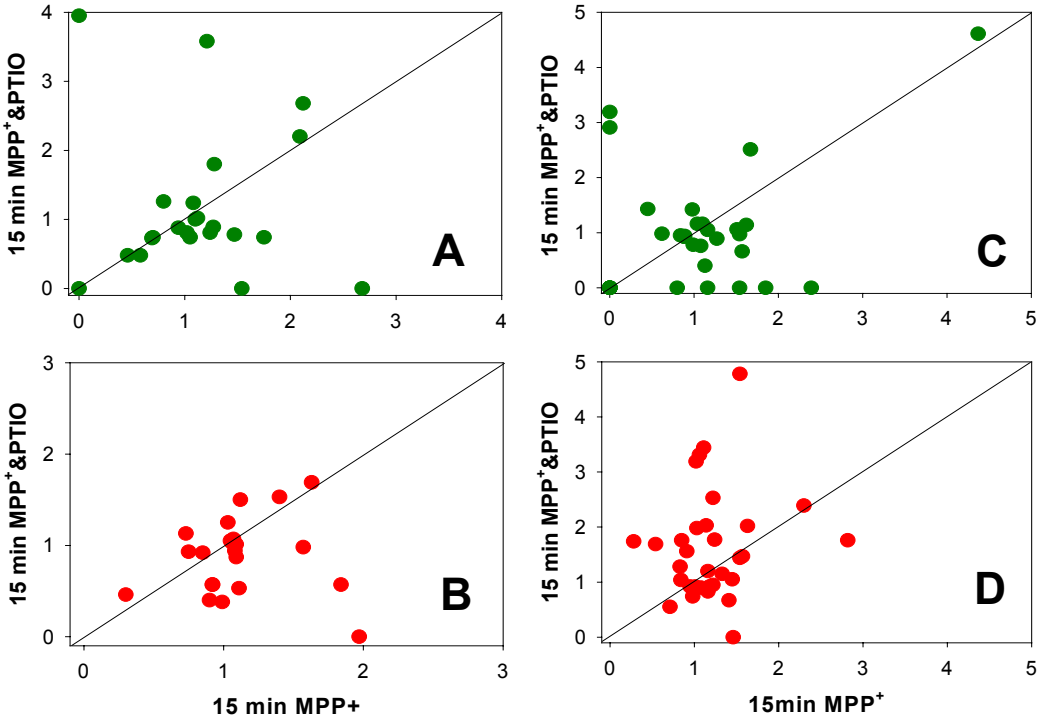
A.



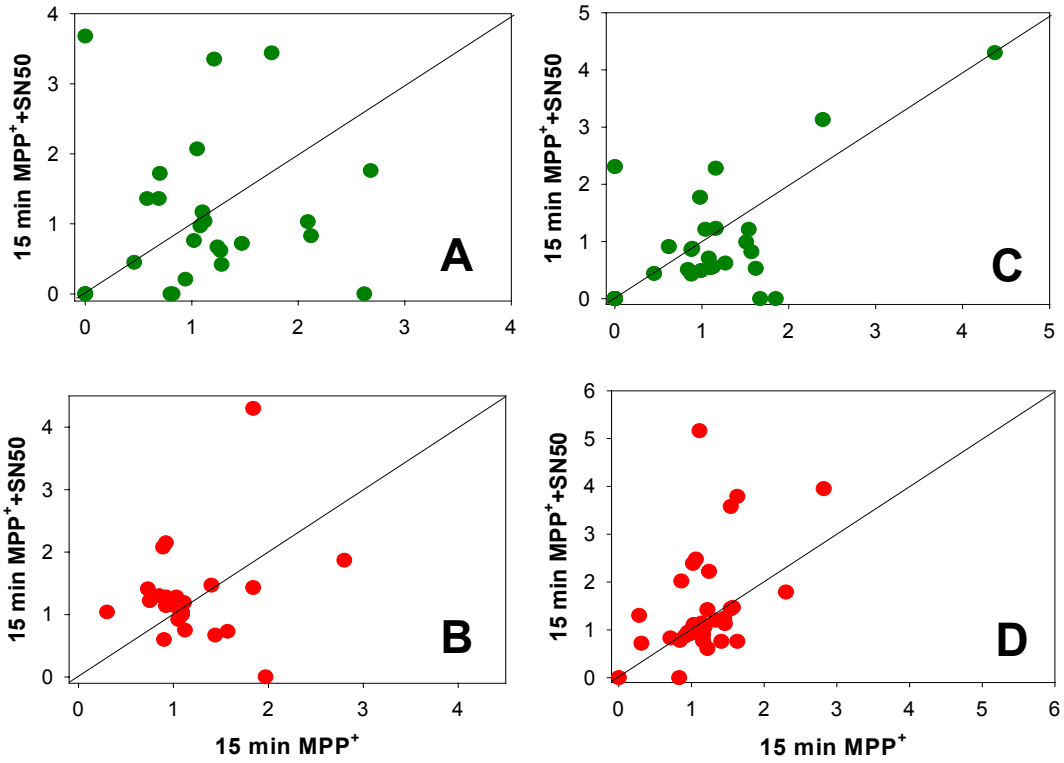
B.



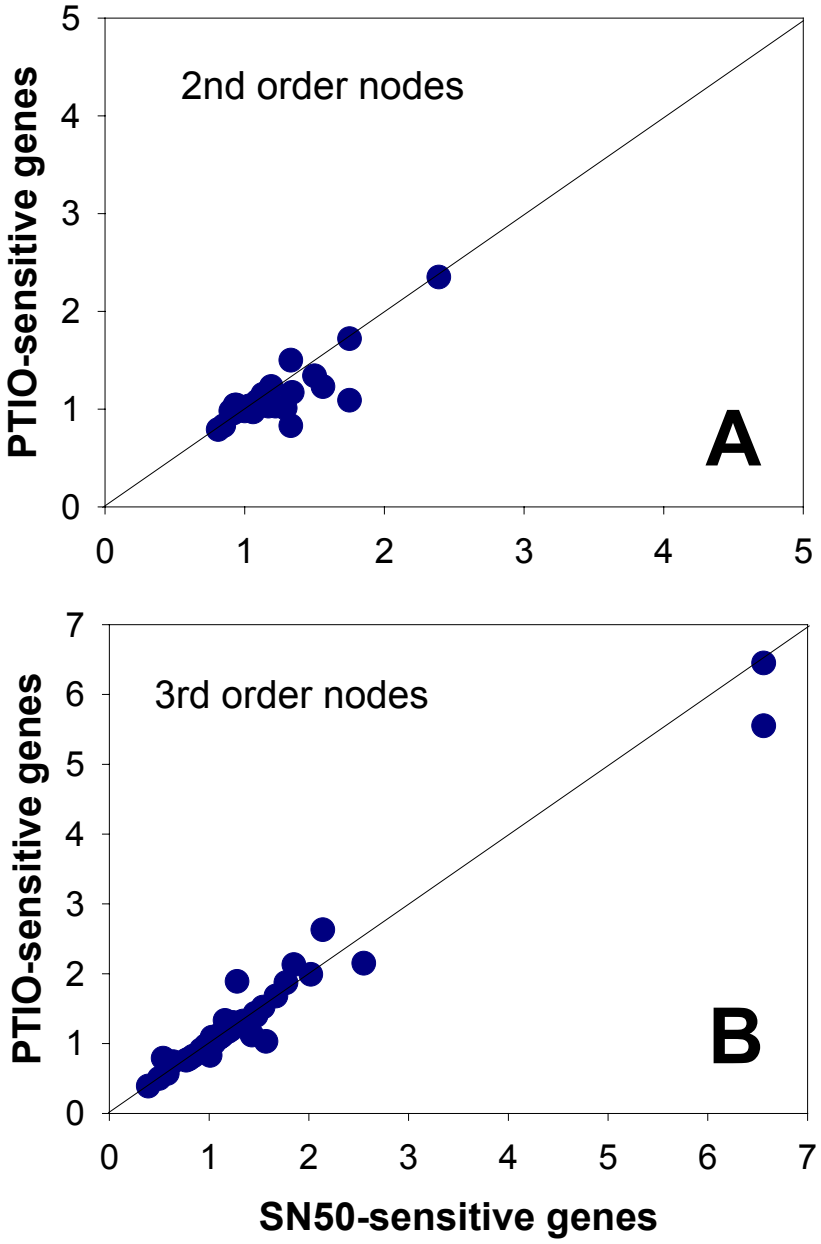
Chapter 3 Figure 8. (A) Area-proportional Venn diagram representing commonalities between genes found to be significantly regulated in SY5Y cells with exposure to 15m MPP+ alone or in combination with PTIO or SN50. (B) Area proportional Venn diagram representing the number of genes significantly regulated at 15m (red) and 90m (blue) of MPP+ exposure in SY5Y cells in relation to the total number of genes assayed by cDNA microarray.



Chapter 3 Figure 9. Correlations between RE values of transcripts grouped into Gene Ontology families by GoMiner software from experiments comparing transcriptosome changes in response to MPP^+ alone to those in response to MPP^+ in presence of the NO scavenger PTIO. RE values for genes downregulated (green) and upregulated (red) are plotted for the second order GoMiner nodes (A,B) and third order nodes (C,D). See Methods for details.



Chapter 3 Figure 10 . Correlations between RE values of transcripts grouped into Gene Ontology families by GoMiner software from experiments comparing transcriptosome changes in response to MPP+ alone to those in response to MPP+ in presence of the NfkappaB antagonist SN50. RE values for genes downregulated (green) and upregulated (red) are plotted for the second order GoMiner nodes (A,B) and third order nodes (C,D). See Methods for details.



Chapter 3 Figure 11. Correlations between RE values of transcripts grouped into Gene Ontology families by GoMiner software from experiments comparing transcriptosome changes for MPP+ altered genes sensitive to blockade by SN50 compared to those sensitive to blockade by PTIO.

Chapter 4

Analysis of brain tissue with varying post mortem intervals: methodological considerations.

One important aspect of the evolution of our work with microarrays is the extension of the experimental paradigm into other model systems. Following our work with nylon arrays utilizing samples derived from our cell model of PD, we were interested in the applicability of our methods to samples that might be more directly indicative of the processes occurring in the disease state. An example of such a sample would be post mortem brain tissue samples from PD and age-matched control patients. Several facilities maintain brain banks comprising anywhere from dozens to thousands of samples from a broad spectrum of neurodegenerative disease patients. A critical issue facing such facilities is the quality of the samples they keep, which is dependent upon multiple factors which certainly include the robustness of the physical plant, quality of curation and the like, but is most directly affected by the method and speed of collection of the tissues at the time of harvest. Post mortem interval (PMI) is a term that refers to the time elapsed between death of the tissue donor and the placement of the tissue under storage conditions. Samples range widely in initial quality, as time elapsed from death to autopsy, and indeed the “agonal period,” or period of energetic/nutritional/trophic compromise to the tissue prior to death.

In isolation of RNA from frozen brain tissue samples archived in the UVA CSND brain bank and in others obtained from a bank curated by Dr. Rajput of the University of Saskatchewan, the quality and yield varied widely. The RNA yield did not appear to depend directly on the PMI of a given sample. In cooperation with Stacey Trotter, a MSTP student performing a summer research rotation in the Bennett lab, we conducted experiments that investigated RNA yield from brain samples from rats after varying PMI. ST performed the animal work and hybridization experiments; LB and JB were jointly responsible for the experimental planning and data analysis, These results were published as a short communication in the journal *Brain Research* in June of 2002.

Postmortem brain tissue is increasingly being used for studying gene expression in neuropsychiatric diseases. RT-PCR, Northern blots, and in situ hybridization all require stable mRNA of high quality (Augood et al, 1999; Benisty et al, 1998; Chen et al, 1999; Dwivedi et al, 2001; Growdon et al, 1999; Osterlund et al, 1999; Marcinkiewicz and Seidah, 2000; Schramm et al, 1999). More recently, gene array technology, allowing the examination of hundreds or thousands of mRNA levels simultaneously, is being used for human postmortem brain studies (Hakak et al, 2001; Lewohl et al, 2000). However, discrepancies in postmortem conditions represent a potential source of significant variation among samples.

Previous studies have focused on testing the quality of mRNA in postmortem brain tissue using different postmortem conditions (Bahn et al, 2001; Barton et al, 1993; Castensson et al, 1993; Gilmore et al, 1993; Harrison et al, 1995; Kingsbury et al, 1995; Leonard et al, 1993; Mathern et al, 1997; Pardue et al, 1994). These studies determined that postmortem interval (time between death and freezing) had little effect upon stability. Some found instead that agonal status, reflected in a decreased brain pH, correlated strongly with a decrease in mRNA stability (Harrison et al, 1995; Kingsbury et al, 1995). Thus far, studies using postmortem brain mRNA are based on the examination of housekeeping genes as well as selected genes of interest. With the advent of gene array technology, it is necessary to revisit the issue of postmortem mRNA stability from a population-wide perspective.

In this study, we examined the effect of postmortem interval (PMI) on mRNA used in gene arrays. Mice were subjected to scenarios that mimic circumstances of postmortem collection of human brains. Our gene arrays indicate that mRNA shows no consistent decline in stability or quality after a PMI of 4 hours at room temperature, followed by overnight refrigeration of the corpse before brain removal. After that, there is an increasing variation in the amount of hybridization of cDNA probe to the gene array membrane, indicating a slight decrease in the stability of isolated mRNA. These findings will be useful for selection of postmortem brain tissue used in experiments involving gene array technology.

Hilltop Balb/C male mice, 25-30 grams were killed using CO_2 asphyxiation. Seven groups of mice with 3 mice per group were created based on postmortem interval:

Group Name	Time spent at room temperature (hours)	Time spent at 4°C (hours)	Total Postmortem Interval (hours)
Immediate	0	0	0
Morgue overnight	0	18	18
RT 4 hours	4	18	22
RT 8 hours	8	18	26
RT 12 hours	12	18	30
RT 24 hours	24	18	42

Once the interval was ended, the brains were dissected, the cerebellum removed, then the forebrain was hemisected. Both halves were placed directly on a block of dry ice. RNA was purified from one half using the RNeasy mini kit (Qiagen). The concentration of RNA was assessed by spectrophotometry. All RNA used had 260/280 ratios of >1.5.

2ug total RNA were used for probe synthesis and hybridization to nylon Atlas Mouse cDNA Expression Arrays according to the manufacturer's protocol (Clontech). cDNA probes were synthesized using [α-^{32}P] dATP (3,000 Ci/mmol). After overnight hybridization and washing, the arrays were exposed to phosphoimager screens. Images were analyzed with Imagene 4.1 software (Biodiscovery).

3 independent brain samples from mice at each postmortem interval were subject to RNA extraction, cDNA labeling and hybridization to genes spotted in duplicate on the membranes. Local background was subtracted from each spot, duplicates were averaged and then normalized first to the mean OD of two standard housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase and cytoplasmic beta-actin). A threshold of at least 10% of the mean housekeeping gene OD was used for establishing detection of a given gene. By this criteria, 365 of the 588 genes arrayed were detected at baseline. Each detected gene was then normalized to the total array OD for all the detected genes on a given array. The three independent values for array-normalized expression of each detected gene were then averaged for comparison across post-mortem conditions.

Postmortem interval conditions were repeated and the entire brain was dissected and the cerebellum removed. The brain was weighed and homogenized in 10 volumes of water. The pH was measured at room temperature using a temperature-compensating pH meter.

Figure 1 shows correlation plots for array-normalized gene expression levels from brains harvested at each postmortem condition, compared to expression levels from brains harvested and frozen immediately. The postmortem conditions of overnight refrigeration ("MG_ON") as well as four hours at room temperature followed by overnight refrigeration ("RT_4HRS") yielded gene expression that correlated highly with that from brains with zero hours PMI and varied with slopes close to unity. More prolonged postmortem conditions caused the correlation to lessen and slopes to drop consistently below unity. Under these conditions more genes were observed to have expression levels both above and below the consensus line.

Figure 2A shows the relationship between baseline level of normalized gene expression and the degree of change found in brains removed after 24 hours at RT/18 hours at 4 degrees. The majority of the 365 detected genes were expressed at relatively low levels. There was no clear relationship among basal levels of expression and degree of change found at the longest postmortem interval examined. Figure 2B shows the population distribution of gene expression indexed to baseline levels. Overall the population distributions were similar, with the longer postmortem intervals showing larger percentages of distribution towards the extremes. However, for all postmortem conditions 90-95% of genes fell within +/- 40% of equivalency. The small number of genes outside of this range was almost all from the later postmortem interval groups.

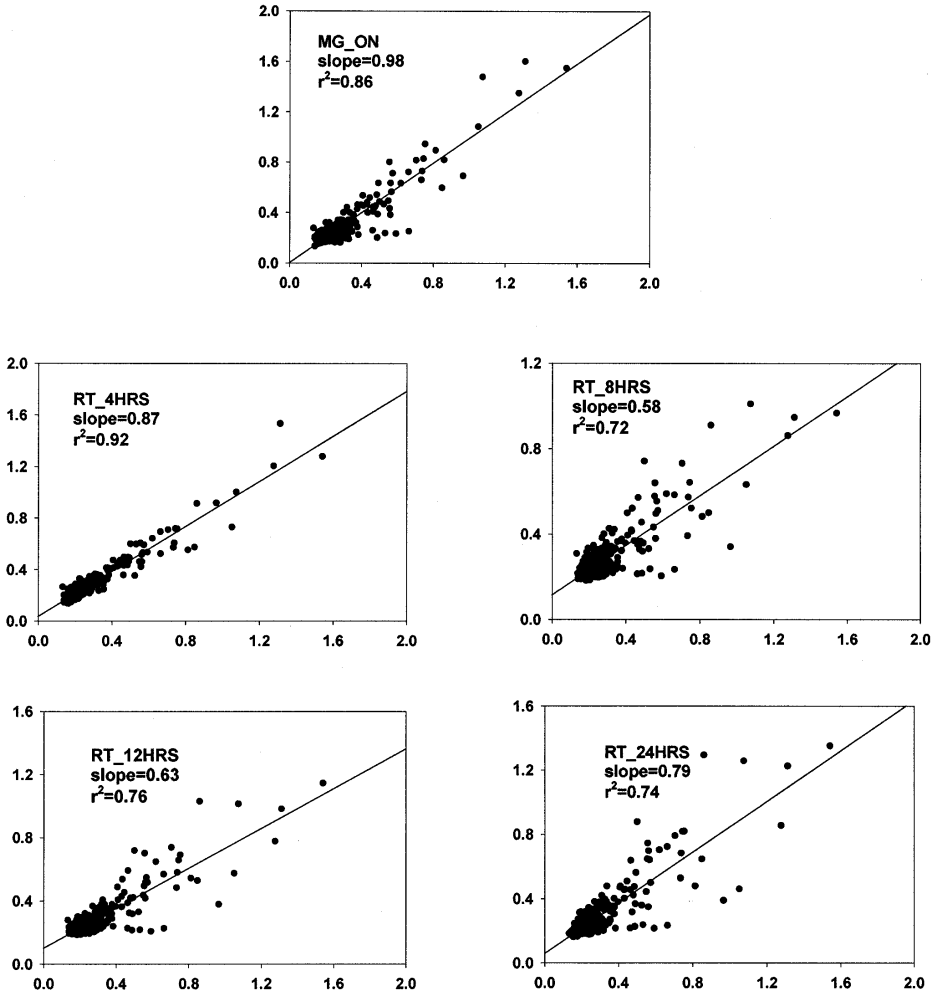
Brain pH was examined for each of the PMI groups and ranged between 6.62 and 7.00 without correlation to any group.

We used low density nylon mouse gene arrays and ^{32}P-labeled cDNA probes to examine postmortem stability of brain mRNAs isolated from mice subjected to varying postmortem conditions. Nylon arrays offer the greatest sensitivity of all available techniques and low cost, but are limited by low gene density as compared to most glass microarrays. Thus, our results must be interpreted within the confines of a limited genomic survey.

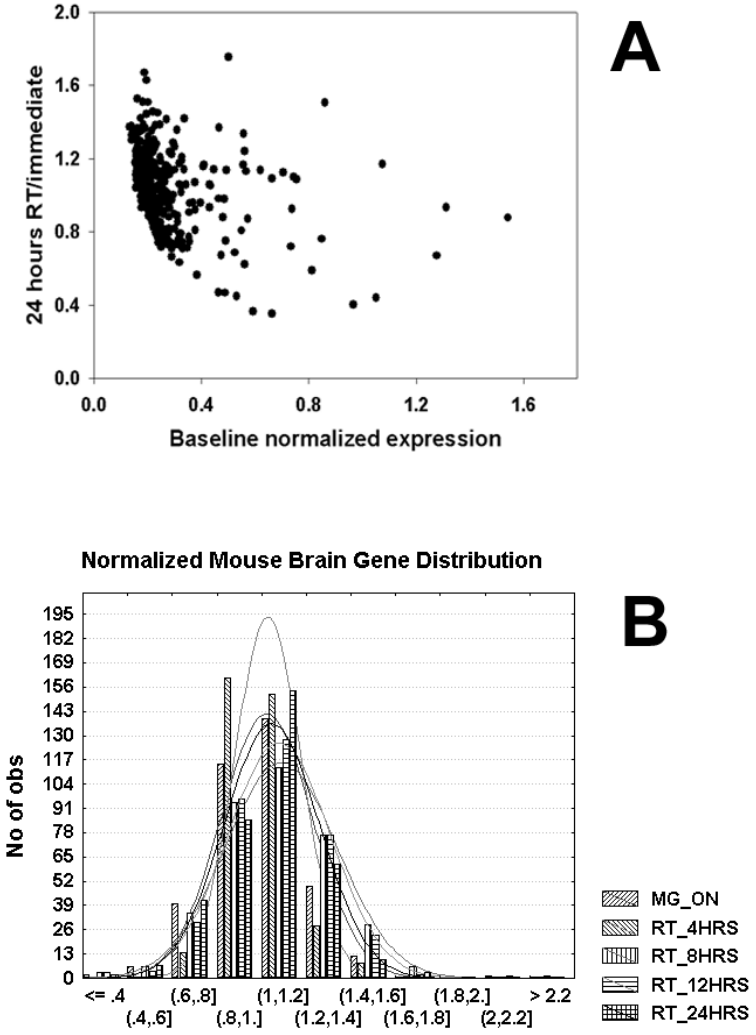
We tried to mimic common scenarios experienced by humans whose brains are harvested for experimental purposes, such as gene array studies. We found that the best correlations with immediate brain harvesting and freezing were in situations of immediate refrigeration or short postmortem time (4 hours) at room temperature. Longer postmortem time at room temperature introduced greater variability in gene expression, but the variation from immediate brain harvesting for most genes was within +/- 40%. This figure is under the 1.5-1.7 fold threshold that many accept for “biologically significant” gene expression changes, although it must be emphasized that any such threshold is arbitrary.

Our preliminary results appear to justify the greater expense associated with carrying out broader genomic surveys of brain tissues harvested under varying postmortem conditions. Because of the trend we observed of increasing postmortem intervals leading to larger populations of genes at the extremes of the distribution curves, we feel such studies should be carried out to determine

the effect of postmortem interval on larger gene population distributions. This type of information would seem essential to interpreting gene array studies of human brain diseases that utilize postmortem brains and should be validated for the arrays used in any particular study.



Chapter 4 Figure 1. Correlations among normalized gene expression from brains removed and frozen immediately (on X axes) and brains removed after different postmortem conditions (on Y axes).



Chapter 4 Figure 2. A. Plot for the 365 detected genes of initial normalized expression level vs. ratio of expression in brains harvested at 24 hours RT/those harvested immediately. B. Histogram plots of population distributions of gene expressions from brains removed after different postmortem conditions and normalized to that of brains removed immediately. A value of 1.0 would indicate the same level of gene expression for a given gene as that found in brains removed immediately after death.

Chapter 5

Conclusions and Future Directions.

My work in the CSND has been a time of great technological and scientific innovation, and the difficulties that I have encountered- technical, statistical, and otherwise, may well be a harbinger of the types of changes that much of biomedical science will be undergoing over the next ten to twenty years. The single greatest innovation since the start of the molecular biology revolution is incontrovertibly the sequencing of the human genome. We are only now taking the first steps in applying this great volume of (unfortunately, largely *un*annotated) knowledge to the practical purpose of treating human disease.

Biologists, as a culture of scientists, are not used to dealing with large, noisy, quantitative data sets such as the type produced by microarray analyses. Certainly the development of enhanced data mining techniques and greater numbers and speed of analyses performed will improve the amount of data collected, but will largely fail to address the issue of quality. As such, I would recommend a substantial investment in the research community to provide a consistent framework, along the lines of a LocusLink-style system, for discussing changes in genes and in proteins in a common language. The need for this is as basic as can be, and will unfortunately require huge numbers of man-hours to accomplish, and therefore large amounts of money. Nevertheless, without this "genetic curacy," the majority of the knowledge accumulated in large scale

studies of genes and proteins may be disjointed and proprietary in its format to the extent that it is unusable by the scientific community at large. This must be avoided at all costs.

With regards to my particular work, I feel that the repeated observation of large nuclear gene expression changes in the face of mitochondrial toxicity represents a new paradigm for approaching the study of mitochondrial pathology as it relates to the greater status of the cell. The data I have presented demonstrates that rapid mitochondrial-nuclear signaling occurs in this cell model, and that it is modulated by different effectors of intracellular signaling. Since mitochondria are known to possess endogenous nitric oxide synthase, and since nitric oxide has recently been demonstrated to impact gene expression through an NFkB dependent mechanism, our data are consistent with nitric oxide being the primary signal indicating mitochondrial distress to the nucleus, and NFkB being the effector molecule that is responsible for the gene expression response that is induced there. Given this notion, it is somewhat surprising to note that treatment with either the NFkB translocation-inhibiting peptide SN50 or the nitric oxide scavenging compound PTIO have no discernible effects upon the course of MPP+ induced cell death. By far the most likely explanation for this phenomenon is that there are multiple pathways in use by the cell to implement such responses, and if one is inhibited, another may be upregulated to cause the same net effect. While this may be somewhat depressing for those of a more reductionistic mindset, I would rather prefer to take it as an endorsement of the efforts that are currently underway to make a more comprehensive catalog of the

interconnections between cellular systems widely available to the scientific community. The preliminary GoMiner data analysis that I have presented in Chapter 3 represents an initial effort in this vein. At present, it is the most comprehensive analysis package of its type available at no cost to the research community at large, and will undoubtedly be improved and refined even as the curation of the genome progresses.

The relevance of these findings to idiopathic Parkinson's disease process *in vivo* is as yet uncertain given our current profound lack of understanding of the interconnections between the various intra and extracellular signaling pathways, but the data here will hopefully provide preliminary clues for which pathways deserve the earliest and closest scrutiny. The RNAi strategies we describe above were my attempt to begin this process; the failure of these experiments was undoubtedly the most disheartening aspect of my time in the CSND, yet I am confident that this and similar approaches represent the best ways to provide direct modulation of the gene expression signals measured by microarray analysis, and that the technical problems that I have encountered are not by any means insurmountable. In addition to RNAi, the question of protein expression/modification changes must be addressed in future experiments. Despite the power of microarrays to measure thousands of RNAs simultaneously, we must be cognizant of the fact that RNA does not exist in a vacuum; many would argue that our efforts would be better served to accelerate completion of a human proteome project, to get to the level of the actuators of molecular activity in the cell. Protein array technology is at least five years

behind DNA array technology today; the collections of antibodies and other binding proteins that are being applied to glass slides for microarray-style analyses are currently few in number, but commercial entities are rapidly improving their offerings in the hope of being first to market. Given resources, I would recommend that any lab group interested in the multiplexed study of gene expression also give serious consideration to construction of protein arrays specific for the model systems they are employing: in the case of the CSND, for example, an array comprising the proteome of dopaminergic nigrostriatal neurons would be highly useful in determining which gene expression responses observed in response to chemical or other perturbations are relevant to the overarching cellular processes of growth, differentiation and death. A cell line with mitochondrial nitric oxide synthase under the control of an inducible repressor would also prove highly useful in beginning to address the question of the contribution of mitochondrial nitric oxide to the cell death process. In this case, as with many research projects, there are indeed many more avenues worthy of investigation than we have the hands, time, and money to pursue as yet.

References

Aarsland D, Tandberg E, Larsen JP, Cummings JL. (1996) Frequency of dementia in Parkinson disease. *Arch Neurol* 53:538-42.

Abe T, Isobe C, Murata T, Sato C, Tohgi H. (2003) Alteration of 8-hydroxyguanosine concentrations in the cerebrospinal fluid and serum from patients with Parkinson's disease. *Neurosci Lett* 336:105-108.

Abramova N. A., Cassarino D. S., Khan S. M., Painter T. W. and Bennett J. P., Jr. (2002) Inhibition by R(+) or S(-) pramipexole of caspase activation and cell death induced by methylpyridinium ion or beta amyloid peptide in SH-SY5Y neuroblastoma. *J. Neurosci. Res*. 67**,** 494-500.

Agid Y, Graybiel AM, Ruberg M, Hirsch E, Blin J, Dubois B, Javoy-Agid F. (1990) The efficacy of levodopa treatment declines in the course of Parkinson's disease: do nondopaminergic lesions play a role? *Adv Neurol* 53:83-100.

Akaike T, Yoshida M, Miyamoto Y, Sato K, Kohno M, Sasamoto K, Miyazaki K, Ueda S, Maeda H. (1993) Antagonistic action of imidazolineoxyl N-oxides against endothelium-derived relaxing factor/.NO through a radical reaction. *Biochemistry* 32:827-32.

Alam ZI, Daniel SE, Lees AJ, Marsden DC, Jenner P, Halliwell B. (1997) A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. *J Neurochem* 69:1326-1329.

Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B. (1997) Oxidative DNA damage in the parkinsonian brain: an apparent

selective increase in 8-hydroxyguanine levels in substantia nigra. *J Neurochem* 69:1196-1203.

Alves Da Costa C, Paitel E, Vincent B, Checler F. (2002) Alpha-synuclein lowers p53-dependent apoptotic response of neuronal cells. Abolishment by 6-hydroxydopamine and implication for Parkinson's disease. *J Biol Chem* 277:50980-50984.

Ambani LM, Van Woert MH, Murphy S. (1975) Brain peroxidase and catalase in Parkinson disease. *Arch Neurol* 32:114-118.

Andringa G, van Oosten RV, Unger W, Hafmans TG, Veening J, Stoof JC, Cools AR. (2000) Systemic administration of the propargylamine CGP 3466B prevents behavioural and morphological deficits in rats with 6-hydroxydopamine-induced lesions in the substantia nigra. *Eur J Neurosci* 12:3033-3043.

Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y. (1997) Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol Histopathol* 12:25-31.

Augood SJ, Waldvogel HJ, Munkle MC, Faull RL, Emson PC, (1999) Localization of calcium-binding proteins and GABA transporter (GAT-1) messenger RNA in the human subthalamic nucleus, *Neuroscience* 88:521-34.

Bahn S, Augood SJ, Ryan M, Standaert DG, Starkey M, Emson PC. (2001) Gene expression profiling in the post-mortem human brain- no cause for dismay. *J Chem Neuroanat* 22:79-94.

Ballard P. A., Tetrud J. W. and Langston J. W. (1985) Permanent human parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): seven cases. *Neurology* **35,** 949–956.

Ballif B. A. and Blenis J. (2001) Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Differ.* 12, 397-408.

Banati RB, Daniel SE, Blunt SB. (1998) Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. *Mov Disord* 13:221-227.

Barone M. V., Crozat A., Tabaee A., Philipson L. and Ron D. (1994) CHOP (GADD153) and its oncogenic variant, TLS-CHOP, have opposing effects on the induction of G1/S arrest. *Genes Dev.* 8:453–464.

Barton AJ, Pearson RC, Najlerahim A, Harrison PJ. (1993) Pre- and postmortem influences on brain RNA. *J Neurochem* 61:1-11.

Barzilai A, Zilkha-Falb R, Daily D, Stern N, Offen D, Ziv I, Melamed E, Shirvan A. (2000) The molecular mechanism of dopamine-induced apoptosis: identification and characterization of genes that mediate dopamine toxicity. *J Neural Transm Suppl* 60:59-76.

Benisty S, Boissiere F, Faucheux B, Agid Y, Hirsch EC. (1998) trkB messenger RNA expression in normal human brain and in the substantia nigra of parkinsonian patients: an in situ hybridization study. *J Neurosci* 86:813-26.

Bertler A, Rosengren E. (1959) Occurrence and distribution of dopamine in brain and other tissues. *Experientia* 15:10-11.

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3:1301-1306.

Beyer W, Imlay J, Fridovich I. (1991) Superoxide dismutases. *Prog Nucleic Acid Res Mol Biol* 40:221-253.

Bindoff LA, Birch-Machin M, Cartilidge NE, Parker WD, Jr, Turnbull DM (1991) Respiratory chain abnormalities in skeletal muscle from patients with Parkinson's disease. *Jour Neurol Sci* 104:203-208.

Birkmayer W, Hornykiewicz O. (1961) Der L-3,4-Dioxyphenylalanin(= DOPA)—Effekt bei der Parkinson-Akinese. *Wien Klin Wochenschr* 3:787-788.

Bolanos JP, Heales SJ, Land JM, Clark JB. (1995) Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary culture. *J Neurochem* 64:1965-1972.

Boveris A, Chance B. (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J* 134:707-716.

Brill LB 2nd, Bennett JP. (2003) Dependence on electron transport chain function and intracellular signaling of genomic responses in SH-SY5Y cells to the mitochondrial neurotoxin MPP+. *Exp Neurol* 181:25-38.

Brookes PS, Salinas EP, Darley-Usmar K, Eiserich JP, Freeman BA, Darley-Usmar VM, Anderson PG. (2000) Concentration-dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome c release. *J Biol Chem* 275:20474-20479.

Brown GC, Borutaite V. (1999) Nitric oxide, cytochrome c and mitochondria. *Biochem Soc Symp* 66:17-25.

Brown VM, Ossadtchi A, Khan AH, Yee S, Lacan G, Melega WP, Cherry SR, Leahy RM, Smith DJ. (2002) Multiplex three-dimensional brain gene expression mapping in a mouse model of Parkinson's disease. *Genome Res* 12:868-884.

Brunk UT, Terman A. (2002) Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic Biol Med* 33:611-619.

Burns R. S., Chiueh C. C., Markey S. P., Ebert M. H., Jacobowitz D. M. and Kopin I. J. (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci U S A* 80:4546–4550.

Candy JM, Perry RH, Perry EK, Irving D, Blessed G, Fairbairn AF, Tomlinson BE. (1983) Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases. *J Neurol Sci* 59:277-89.

Carlsson A, Lindqvist M, Magnusson T, Waldeck B. (1958) On the presence of 3-hydroxytyramine in brain. *Science* 127:471.

Cassarino DS, Bennett JP Jr. (1999) An evaluation of the role of mitochondria in neurodegenerative diseases: mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. *Brain Res Brain Res Rev* 1, 1-25.

Cassarino DS, Fall CP, Swerdlow RH, Smith TS, Halvorsen EM, Miller SW, Parks JP, Parker WD Jr, and Bennett JP Jr. (1997) Elevated reactive

oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease. *Biochim. Biophys Acta* 1362, 77–86.

Cassarino DS, Halvorsen EM, Swerdlow RH, Abramova NN, Parker WD Jr, Sturgill TW, Bennett JP Jr. (2000) Interaction among mitochondria, mitogen-activated protein kinases, and nuclear factor-kappaB in cellular models of Parkinson's disease. *J Neurochem* 74:1384-1392.

Castensson A, Emilsson L, Preece P, Jazin E. (2000) High-resolution quantification of specific mRNA levels in human brain autopsies and biopsies. *Genome Res* 10:1219-1229.

Chance B, Schoener B, Oshino R, Itshak F, Nakase Y. (1979) Oxidation-reduction ratio studies of mitochondria in freeze-trapped samples. NADH and flavoprotein fluorescence signals. *J Biol Chem* 254:4764-4771.

Charcot JM, Vulpian A. (1861) De la paralysie agitante. *Gaz Hebdom Med Chir* 8:765-767.

Chen L, Segal DM, Mash DC. (1999) Semi-quantitative reverse-transcriptase polymerase chain reaction: an approach for the measurement of target gene expression in human brain. Brain Res Brain Res Prot 4:132-139.

Chiba K, Trevor A, Castagnoli N. (1984) Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun* 120, 547–578.

Cogoni C, Irelan JT, Schumacher M, Schmidhauser TJ, Selker EU, Macino G. (1996) Transgene silencing of the al-1 gene in vegetative cells of

Neurospora is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation. *EMBO J* 15:3153-3163.

Conn KJ, Gao WW, Ullman MD, McKeon-O'Malley C, Eisenhauer PB, Fine RE, Wells JM. (2002) Specific up-regulation of GADD153/CHOP in 1-methyl-4-phenyl-pyridinium-treated SH-SY5Y cells. J Neurosci Res 68:755-760.

Cotzias GC, Papavasiliou PS, Gellene R. (1967) Modification of Parkinson's disease—Chronic treatment with L-dopa. *N Engl J Med* 280:337-345.

Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, Kopin IJ. (1979) Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Res* 1(3:249-254.

Dehmer T, Lindenau J, Haid S, Dichgans J, Schulz JB. (2000) Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem* 74:2213-2216.

Dennis J, Bennett JP Jr. (2003) Interactions among nitric oxide and Bcl-family proteins after MPP+ exposure of SH-SY5Y neural cells II: exogenous NO replicates MPP+ actions. *J Neurosci Res* 72:89-97.

Dexter DT, Carayon A, Vidailhet M, Ruberg M, Agid F, Agid Y, Lees AJ, Wells FR, Jenner P, Marsden CD. (1990) Decreased ferritin levels in brain in Parkinson's disease. *J Neurochem* 55:16-20.

Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD. (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* 52:381-389.

Dexter DT, Wells FR, Lees AJ, Agid F, Agid Y, Jenner P, Marsden CD. (1989) Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J Neurochem* 52:1830-1836.

Di Monte DA, Lavasani M, Manning-Bog AB. (2002) Environmental factors in Parkinson's disease. *Neurotoxicology* 23:487-502.

Duvoisin RC. (1965) A review of drug therapy in parkinsonism. *Bull NY Acad Med* 41:898-910.

Dwivedi Y, Rizavi HS, Roberts RC, Conley RC, Tamminga CA, Pandey GN. (2001) Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide patients, *J Neurochem* 77:916-928.

Ehringer H, Hornykiewicz O. (1960) Veteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. *Klin Wochenschr* 38:1236-1239.

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411:494-498.

Fall CP, Bennett JP Jr. (1999) Characterization and time course of MPP+ -induced apoptosis in human SH-SY5Y neuroblastoma cells. *J Neurosci Res* 55:620-628.

Fahn S, Cohen G. (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neurol* 32:804-812.

Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, Copeland RA, Magolda RL,

Scherle PA, Trzaskos JM. (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem* 273, 18623–18632.

Fawcett TW, Eastman HB, Martindale JL, Holbrook NJ. (1996) Physical and functional association between GADD153 and CCAAT/enhancer-binding protein beta during cellular stress. *J Biol Chem* 271**,** 14285‑14289.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 391:806-811.

Frangiskakis JM, Ewart AK, Morris CA, Mervis CB, Bertrand J, Robinson BF, Klein BP, Ensing GJ, Everett LA, Green ED, Proschel C, Gutowski NJ, Noble M, Atkinson DL, Odelberg SJ, Keating MT. (1996) LIM-kinase1 hemizygosity implicated in impaired visuospatial constructive cognition. *Cell* 86:59-69.

Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S. (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 344(10):710-719.

Gabby M, Tauber M, Porat S, Simantov R. (1996) Selective role of glutathione in protecting human neuronal cells from dopamine-induced apoptosis. *Neuropharmacology* 35:571-578.

Gardner PR, Raineri I, Epstein LB, White CW. (1995) Superoxide radical and iron modulate aconitase activity in mammalian cells. *J Biol Chem* 270:13399-13405.

Gilmore JH, Lawler CP, Eaton AM, Mailman RB. (1993) Postmortem stability of dopamine D1 receptor mRNA and D1 receptors. *Brain Res Molec Brain Res* 18:290-296.

Good PF, Hsu A, Werner P, Perl DP, Olanow CW. (1998) Protein nitration in Parkinson's disease. *J Neuropathol Exp Neurol* 57:338-342.

Gowers WR. (1893) *A manual of diseases of the nervous system, vol. 2*: Philadelphia, Blakiston. 636-657.

Graeber MB, Moran LB. (2002) Mechanisms of cell death in neurodegenerative diseases: fashion, fiction, and facts. *Brain Pathol* 12:385-390.

Greenfield JG, Bosanquet FD. (1953) The brain-stem lesions in Parkinsonism. *J Neurochem* 16:213-26.

Growdon WB, Cheung BS, Hyman BT, Rebeck GW. (1999) Lack of allelic imbalance in APOE epsilon3/4 brain mRNA expression in Alzheimer's disease, *Neurosci Lett* 272:83-86.

Grunewald T, Beal MF. (1999) NOS knockouts and neuroprotection. *Nat Med* 5:1354-1355.

Guo S, Kemphues KJ. (1995) par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* 81:611-620.

Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, Haroutunian V, Fienberg AA. (2001) Genome-wide expression analysis reveals

dysregulation of myelination-related genes in chronic schizophrenia. *Proc Nat Acad Sci U S A* 98:4746-4751.

Haley B, Tang G, Zamore PD. (2003) In vitro analysis of RNA interference in Drosophila melanogaster. *Methods* 30:330-336.

Halvorsen EM, Dennis J, Keeney P, Sturgill TW, Tuttle JB, Bennett JB Jr. (2002) Methylpyridinium (MPP(+))- and nerve growth factor-induced changes in pro- and anti-apoptotic signaling pathways in SH-SY5Y neuroblastoma cells. *Brain Res* 952:98-110.

Hantraye P, Brouillet E, Ferrante R, Palfi S, Dolan R, Matthews RT, Beal MF. (1996) Inhibition of neuronal nitric oxide synthase prevents MPTP-induced parkinsonism in baboons. *Nat Med* 2:1017-1021.

Harrison PJ, Heath PR, Eastwood SL, Burnet PW, McDonald B, Pearson RC. (1995) The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: selective mRNA vulnerability and comparison with their encoded proteins. *Neurosci Lett* 200:151-154.

Hartmann A, Hunot S, Michel PP, Muriel MP, Vyas S, Faucheux BA, Mouatt-Prigent A, Turmel H, Srinivasan A, Ruberg M, Evan GI, Agid Y, Hirsch EC. (2000) Caspase-3: A vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proc Natl Acad Sci U S A* 97:2875-2880.

Hasegawa E, Takeshige K, Oishi T, Murai Y, Minakami S. (1990) 1-Methyl-4-phenylpyridinium (MPP+) induces NADH-dependent superoxide

formation and enhances NADH-dependent lipid peroxidation in bovine heart submitochondrial particles. *Biochem Biophys Res Commun* 170:1049-1055.

Heikkila RE, Manzino L, Cabbat FS, Duvoisin RC. (1984) Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature* 311:467-469.

Hirsch E, Graybiel AM, Agid YA. (1988) Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature* 6180:345-8.

Hirsch EC, Brandel JP, Galle P, Javoy-Agid F, Agid Y. (1991) Iron and aluminum increase in the substantia nigra of patients with Parkinson's disease: an X-ray microanalysis. *J Neurochem* 56:446-451.

Hunot S, Boissiere F, Faucheux B, Brugg B, Mouatt-Prigent A, Agid Y, Hirsch EC. (1996) Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* 72:355-363.

Ischiropoulos H, al-Mehdi AB. (1995) Peroxynitrite-mediated oxidative protein modifications. *FEBS Lett* 364:279-282.

Itzhak Y, Martin JL, Ali SF. (1999) Methamphetamine- and 1-methyl-4-phenyl- 1,2,3, 6-tetrahydropyridine-induced dopaminergic neurotoxicity in inducible nitric oxide synthase-deficient mice. *Synapse* 34:305-312.

Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH. (1985) Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 - tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by

dopamine neurons explains selective toxicity. *Proc Natl Acad Sci U S A* 82:2173-2177.

Jellinger K, Kienzl E, Rumpelmair G, Riederer P, Stachelberger H, Ben-Shachar D, Youdim MB. (1992) Iron-melanin complex in substantia nigra of parkinsonian brains: an x-ray microanalysis. *J Neurochem* 59:1168-1171.

Jellinger KA, Stadelmann C. (2000) Mechanisms of cell death in neurodegenerative disorders. *J Neural Transm Suppl* 59:95-114.

Jenner P, Rupniak NM, Rose S, Kelly E, Kilpatrick G, Lees A, Marsden CD. (1984) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. *Neurosci Lett* 50:85–90.

Jorgensen RA, Cluster PD, English J, Que Q, Napoli CA. (1996) Chalcone synthase cosuppression phenotypes in petunia flowers: comparison of sense vs. antisense constructs and single-copy vs. complex T-DNA sequences. *Plant Mol Biol* 31:957-973.

Ju C, Yoon KN, Oh YK, Kim HC, Shin CY, Ryu JR, Ko KH, Kim WK. (2000) Synergistic depletion of astrocytic glutathione by glucose deprivation and peroxynitrite: correlation with mitochondrial dysfunction and subsequent cell death. *J Neurochem* 74:1989-1998.

Junn E, Mouradian MM. (2001) Apoptotic signaling in dopamine-induced cell death: the role of oxidative stress, p38 mitogen-activated protein kinase, cytochrome c and caspases. *J Neurochem* 78:374-383.

Kikuchi A, Takeda A, Onodera H, Kimpara T, Hisanaga K, Sato N, Nunomura A, Castellani RJ, Perry G, Smith MA, Itoyama Y. (2002) Systemic

increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiol Dis* 9:244-248.

Kingsbury AE, Foster OJ, Nisbet AP, Cairns N, Bray L, Eve DJ, Lees AJ, Marsden CD. (1995) Tissue pH as an indicator of mRNA preservation in human post-mortem brain. *Brain Res Molec Brain Res* 28:311-318.

Kish SJ, Morito C, Hornykiewicz O. (1985) Glutathione peroxidase activity in Parkinson's disease brain. *Neurosci Lett* 58:343-346.

Kuo WP, Jenssen TK, Butte AJ, Ohno-Machado L, Kohane IS. (2002) Analysis of matched mRNA measurements from two different microarray technologies. *Bioinformatics* 18:405-412.

Langston JW, Ballard P, Tetrud JW, Irwin I. (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219:979-980.

Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D. (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Ann Neurol* 46:598-605.

Langston JW, Langston EB, Irwin I. (1984) MPTP-induced parkinsonism in human and non-human primates--clinical and experimental aspects. *Acta Neurol Scand Suppl* 100, 49–54.

Lebovitz RM, Zhang H, Vogel H, Cartwright J Jr, Dionne L, Lu N, Huang S, Matzuk MM. (1996) Neurodegeneration, myocardial injury, and perinatal death

in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci U S A* 93:9782-9787.

Lee EH, Yang YL, Chia LG, Kuo JS. (1994) Interactions among deprenyl, clorgyline and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on catecholamines in mice. *Neurosci Lett* 165:149-52.

Lee R, Kermani P, Teng KK, Hempstead BL. (2001) Regulation of cell survival by secreted proneurotrophins. *Science* 294:1945-1948.

Leonard S, Logel J, Luthman D, Casanova M, Kirch D, Freedman R. (1993) Biological stability of mRNA isolated from human postmortem brain collections. *Biol Psychiatry* 33:456-466.

Leveugle B, Faucheux BA, Bouras C, Nillesse N, Spik G, Hirsch EC, Agid Y, Hof PR. (1996) Cellular distribution of the iron-binding protein lactotransferrin in the mesencephalon of Parkinson's disease cases. *Acta Neuropathol (Berl)* 91:566-572.

Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA. (2000) Gene expression in human alcoholism: microarray analysis of frontal cortex. *Alcoholism Clin Exp Res* 24:1873-1882.

Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, et al. (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 11:376-381.

Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, Dawson VL, Dawson TM, Przedborski S. (1999) Inducible nitric

oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. *Nat Med* 5:1403-1409.

Lin YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J. (1995) Inhibition of nuclear translocation of transcription factor NF-kappa B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *J Biol Chem* 270:14255-8.

Lucking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Denefle P, Wood NW, Agid Y, Brice A. (2000) Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. *N Engl J Med* 342:1560-7.

Luethy JD, Holbrook NJ. (1992) Activation of the gadd153 promoter by genotoxic agents: a rapid and specific response to DNA damage. *Cancer Res* 52:5–10.

Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K, Narumiya S. (1999) Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* 285:895-898.

Manche L, Green SR, Schmedt C, Mathews MB. (1992) Interactions between double-stranded RNA regulators and the protein kinase DAI. *Mol Cell Biol* 12:5238-5248.

Mandel S, Grunblatt E, Maor G, Youdim MB. (2002) Early and late gene changes in MPTP mice model of Parkinson's disease employing cDNA microarray. *Neurochem Res* 27:1231-1243.

Mandir AS, Przedborski S, Jackson-Lewis V, Wang ZQ, Simbulan-Rosenthal CM, Smulson ME, Hoffman BE, Guastella DB, Dawson VL, Dawson TM. (1999) Poly(ADP-ribose) polymerase activation mediates 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism. *Proc Natl Acad Sci U S A* 96:5774-5779.

Mann VM, Cooper JM, Kriige D, Daniel SE, Schapira AHV, Marsden D (1992) Brain, skeletal muscle, and platelet homogenate mitochondrial functions in Parkinson's disease. *Brain* 115:333-342.

Marcinkiewicz M, Seidah NG. (2000) Coordinated expression of beta-amyloid precursor protein and the putative beta-secretase BACE and alpha-secretase ADAM10 in mouse and human brain. *J Neurochem* 75:2133-2143.

Masserano JM, Gong L, Kulaga H, Baker I, Wyatt RJ. (1996) Dopamine induces apoptotic cell death of a catecholaminergic cell line derived from the central nervous system. *Mol Pharmacol* 50:1309-1315.

Mathern GW, Pretorius JK, Kornblum HI, Mendoza D, Lozada A, Leite JP, Chimelli MC, Fried I, Sakamoto AC, Assirati JA, Levesque MF, Adelson PD, Peacock WJ. (1997) Human hippocampal AMPA and NMDA mRNA levels in temporal lobe epilepsy patients. *Brain* 120:1937-1959.

Matsumoto M, Minami M, Takeda K, Sakao Y, Akira S. (1996) Ectopic expression of CHOP (GADD153) induces apoptosis in M1 myeloblastic leukemia cells. *FEBS Lett* 395:143–147.

Matthews RT, Yang L, Beal MF. (1997) S-Methylthiocitrulline, a neuronal nitric oxide synthase inhibitor, protects against malonate and MPTP neurotoxicity. *Exp Neurol* 143:282-286.

Melov S, Schneider JA, Day BJ, Hinerfeld D, Coskun P, Mirra SS, Crapo JD, Wallace DC. (1998) A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nat Genet* 18:159-163.

Merritt HH (1956) Paralysis agitans [editorial]. *J Chron Dis* 6:654-657.

Minks MA, Benvin S, Maroney PA, Baglioni C. (1979) Metabolic stability of 2' 5'oligo (A) and activity of 2' 5'oligo (A)-dependent endonuclease in extracts of interferon-treated and control HeLa cells. *Nucleic Acids Res* 6:767-780.

Mizuno Y, Ohta S, Tanaka M, Takamiya S, Suzuki K, Sato T, Oya H, Ozawa T, and Kagawa Y (1989) Deficiencies of the respiratory chain in Parkinson's disease. *Biochem Biophys Res Comm* 163:1450-1455.

Mochizuki H, Goto K, Mori H, Mizuno Y. (1996) Histochemical detection of apoptosis in Parkinson's disease. *J Neurol Sci* 137:120-123.

Montagu KA. (1957) Catechol compounds in rat tissues and in brains of different animals. *Nature* 180:244-245.

Moresco RM, Volonte MA, Messa C, Gobbo C, Galli L, Carpinelli A, Rizzo G, Panzacchi A, Franceschi M, Fazio F. (2002) New perspectives on neurochemical effects of amantadine in the brain of parkinsonian patients: a PET - [(11)C]raclopride study. *J Neural Transm* 109:1265-74.

Mytilineau C, Werner P, Molinari S, DiRocco A, Cohen G, Yahr MD (1994) Impaired oxidative decarboxylation of pyruvate in fibroblasts from patients with Parkinson's disease. *J Neural Transm-Park Dis Dement Sect* 8: 223-228.

Nakayama, K. (1997) Furin: a mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins. *Biochem J* :625–635.

Napolitano M, Centonze D, Calce A, Picconi B, Spiezia S, Gulino A, Bernardi G, Calabresi P. (2002) Experimental parkinsonism modulates multiple genes involved in the transduction of dopaminergic signals in the striatum. *Neurobiol Dis* 10:387-395.

Nicklas WJ, Vyas I, Heikkila RE. (1985) Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sci* 8:223–228.

Offen D, Beart PM, Cheung NS, Pascoe CJ, Hochman A, Gorodin S, Melamed E, Bernard R, Bernard O. (1998) Transgenic mice expressing human Bcl-2 in their neurons are resistant to 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine neurotoxicity. *Proc Natl Acad Sci U S A* 95:5789-5794.

Offen D, Ziv I, Panet H, Wasserman L, Stein R, Melamed E, Barzilai A. (1997) Dopamine-induced apoptosis is inhibited in PC12 cells expressing Bcl-2. *Cell Mol Neurobiol* 17:289-304.

Olanow CW. (2002) Surgical therapy for Parkinson's disease. *Eur J Neurol* 3:31-39.

Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP, Godbold J, Freeman TB. (2003) A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol* 54:403-14.

Oo TF, Siman R, Burke RE. (2002) Distinct nuclear and cytoplasmic localization of caspase cleavage products in two models of induced apoptotic death in dopamine neurons of the substantia nigra. *Exp Neurol* 175:1-9.

Osterlund MK, Keller E, Hurd YL. (2000) The human forebrain has discrete estrogen receptor alpha messenger RNA expression: high levels in the amygdaloid complex. *Neuroscience* 95:333-342.

Pardue S, Zimmerman AL, Morrison-Bogorad M. (1994) Selective postmortem degradation of inducible heat shock protein 70 (hsp70) mRNAs in rat brain. *Cell Mol Neurobio* 14:341-357.

Parker WD Jr, Boyson SJ, Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson’s disease. *Ann Neurol* 26:719-723.

Parkinson J. (1817) *An essay on the shaking palsy*. London, Whittingham and Rowland for Sherwood, Neely, Jones.

Pearce RK, Owen A, Daniel S, Jenner P, Marsden CD. (1997) Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. *J Neural Transm* 104:661-677.

Perry TL, Godin DV, Hansen S. (1982) Parkinson's disease: a disorder due to nigral glutathione deficiency? *Neurosci Lett* 33:305-310.

Peterson F. (1890) Paralysis agitans. In: Starr MA, ed., *Familiar Forms of Nervous Disease.* New York, Columbia University Press.

Poewe W, Wenning G. (2002) The differential diagnosis of Parkinson's disease. *Eur J Neurol* 9 Suppl 3:23-30.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045-2047.

Pryor WA. (1986) Oxy-radicals and related species: their formation, lifetimes, and reactions. *Ann Rev Physiol* 48:657-667.

Przedborski S, Jackson-Lewis V, Yokoyama R, Shibata T, Dawson VL, Dawson TM. (1996) Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. *Proc Natl Acad Sci U S A* 93:4565-4571.

Przedborski S, Kostic V, Jackson-Lewis V, Naini AB, Simonetti S, Fahn S, Carlson E, Epstein CJ, Cadet JL. (1992) Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *J Neurosci* 12:1658-1667.

Qiao D, Im E, Qi W, Martinez JD. (2002) Activator protein-1 and CCAAT/enhancer-binding protein mediated GADD153 expression is involved in deoxycholic acid-induced apoptosis. *Biochim Biophys Acta* 1583:108-16.

Raha S, McEachern GE, Myint AT, Robinson BH. (2000) Superoxides from mitochondrial complex III: the role of manganese superoxide dismutase. *Free Radic Biol Med* 29:170-180.

Riederer P, Sofic E, Rausch WD, Schmidt B, Reynolds GP, Jellinger K, Youdim MB. (1989) Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. *J Neurochem* 52:515-520.

Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, Tung KH, Tanner CM, Masaki KH, Blanchette PL, Curb JD, Popper JS, White LR. (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA* 283:2674-2679.

Sano I, Gamo T, Kakimoto Y, Taniguchi K, Takesada M, Nishinuma K. (1959) Distribution of catechol compounds in human brain. *Biochem Biophys Acta* 32:586-587.

Schramm M, Falkai P, Tepest R, Schneider-Axmann T, Przkora R, Waha A, Pietsch T, Bonte W, Bayer TA. (1999) Stability of RNA transcripts in post-mortem psychiatric brains. *J Neur Tran* 106:329-335.

Schulz JB, Matthews RT, Muqit MM, Browne SE, Beal MF. (1995) Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J Neurochem* 64:936-939.

Schwab RS, England AC Jr., Poskanzer DC, Young RR. (1969) Amantadine in the treatment of Parkinson's disease. *JAMA* 208:1168-1170.

Snyder SH, D'Amato RJ. (1986) MPTP: a neurotoxin relevant to the pathophysiology of Parkinson's disease. The 1985 George C. Cotzias lecture. *Neurology* 36:250–258.

Sofic E, Lange KW, Jellinger K, Riederer P. (1992) Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. *Neurosci Lett* 142:128-130.

Stephans SE, Miller GW, Levey AI, Greenamyre JT (2002) Acute mitochondrial and chronic toxicological effects of 1-methyl-4-phenylpyridinium in human neuroblastoma cells. *Neurotoxicol* 23:569-580.

Sveinbjornsdottir S, Hicks AA, Jonsson T, Petursson H, Gugmundsson G, Frigge ML, Kong A, Gulcher JR, Stefansson K. (2000) Familial aggregation of Parkinson's disease in Iceland. *N Engl J Med* 343:1765-70.

Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA**,** Sheehan JP, Bennett JP Jr, Davis RE, Parker WD Jr. (1996) Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol* 40:663-671.

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW. Parkinson disease in twins: an etiologic study. (1999) *JAMA* 281:341-6.

Timmons L, Court DL, Fire A. (2001) Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. *Gene* 263:103-112.

Trimmer PA, Smith TS, Jung AB, Bennett JP Jr. (1996) Dopamine neurons from transgenic mice with a knockout of the p53 gene resist MPTP neurotoxicity. *Neurodegeneration* 5:233-239.

Turrens JF, Alexandre A, Lehninger AL. (1985) Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch Biochem Biophys* 237:408-414.

Veech GA, Dennis J, Keeney PM, Fall CP, Swerdlow RH, Parker WD Jr, Bennett JP Jr. (2000) Disrupted mitochondrial electron transport function increases expression of anti-apoptotic bcl-2 and bcl-X(L) proteins in SH-SY5Y neuroblastoma and in Parkinson disease cybrid cells through oxidative stress. *J Neurosci Res* 61:693-700.

Vila M, Jackson-Lewis VV, Vukosavic S, Djaldetti R, Liberatore G, Offen D, Korsmeyer SJ, Przedborski S. (2001) Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A* 98:2837-2842.

Viswanath V, Wu Y, Boonplueang R, Chen S, Stevenson FF, Yantiri F, Yang L, Beal MF, Andersen JK. (2001) Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease. J Neurosci 21:9519-9528.

Vlahos CJ, Matter WF, Hui KY, Brown RF. (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 269:5241-5248.

Von Economo, C. (1931) The sequelae of encephalitis lethargica. In: Newman K., ed., *Encephalitis lethargica its sequelae and treatment*: 105-156.

Waldmeier PC, Spooren WP, Hengerer B. (2000) CGP 3466 protects dopaminergic neurons in lesion models of Parkinson's disease. *Naunyn Schmiedebergs Arch Pharmacol* 362:526-537.

Yang L, Matthews RT, Schulz JB, Klockgether T, Liao AW, Martinou JC, Penney JB Jr, Hyman BT, Beal MF. (1998) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyride neurotoxicity is attenuated in mice overexpressing Bcl-2. *J Neurosci* 18:8145-8152.

Yoo MS, Chun HS, Son JJ, DeGiorgio LA, Kim DJ, Peng C, Son JH. (2003) Oxidative stress regulated genes in nigral dopaminergic neuronal cells: correlation with the known pathology in Parkinson's disease. *Brain Res Mol Brain Res* 110:76-84.

Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y. (1996) Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. *Proc Natl Acad Sci U S A* 93:2696-2701.

Zeeberg BR, Feng W, Wang G, Wang MD, Fojo AT, Sunshine M, Narasimhan S, Kane DW, Reinhold WC, Lababidi S, Bussey KJ, Riss J, Barrett JC, Weinstein JN. (2003) GoMiner: a resource for biological interpretation of genomic and proteomic data. *Genome Biol* 4:R28.

Zhang J, Perry G, Smith MA, Robertson D, Olson SJ, Graham DG, Montine TJ. (1999) Parkinson's disease is associated with oxidative damage to

cytoplasmic DNA and RNA in substantia nigra neurons. *Am J Pathol* 154:1423-1429.

Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens J L, Ron D. (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev* 12:982–995.

Ziv I, Melamed E, Nardi N, Luria D, Achiron A, Offen D, Barzilai A. (1994) Dopamine induces apoptosis-like cell death in cultured chick sympathetic neurons--a possible novel pathogenetic mechanism in Parkinson's disease. *Neurosci Lett* 170:136-140.

Ziv I, Offen D, Barzilai A, Haviv R, Stein R, Zilkha-Falb R, Shirvan A, Melamed E. (1997) Modulation of control mechanisms of dopamine-induced apoptosis--a future approach to the treatment of Parkinson's disease? *J Neural Transm Suppl* 49:195-202.

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Appendix A. Genes that change +/- 2 fold in response to 15m MPP+.

GENBANK	+/- FOLD	SYMBOL	LOCUSLINK	GENENAME	GENE ONTOLOGY
H47026	2.10507	MGAT3	4248	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	Golgi apparatus; N-linked glycosylation; beta-1,4-mannosylglycoprotein beta-1,4-N-acetylglucosaminyltransferase activity; integral to membrane; transferase activity, transferring glycosyl groups
R19118	2.10065	SDCBP	6386	syndecan binding protein (syntenin)	actin modulating activity; adherens junction; cytoskeletal adaptor activity; cytoskeleton; endoplasmic reticulum; interleukin-5 receptor binding; interleukin-5 receptor complex; intracellular signaling cascade; membrane; neurexin binding; nucleus; protein-membrane targeting; regulation of synapse; substrate-bound cell migration, cell extension; syndecan binding
H26760	2.02783	KIAA0375	9853	KIAA0375 gene product	
T77387	1.99795				
H67530	1.88371	MYH11	4629	myosin, heavy polypeptide 11, smooth muscle	ATP binding; actin binding; calmodulin binding; cell growth and/or maintenance; kinesin complex; motor activity; muscle development; muscle myosin; myosin; striated muscle contraction; striated muscle thick filament

R17538	1.84664	SYN2	6854	Synapsin 2	Neuronal phosphoproteins; synaptic proteins; neurotransmission; nucleus
N93442	1.81151	TTC11	51024	tetratricopeptide repeat domain 11	
R44837	1.76474		339479	similar to RIKEN cDNA B830045N13	
H47146	1.70916	ERCC1	2067	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	DNA repair; embryogenesis and morphogenesis; endodeoxyribonuclease activity; nucleotide-excision repair; nucleus
H83405	1.69136	FGD1	2245	faciogenital dysplasia (Aarskog-Scott syndrome)	development; guanyl-nucleotide exchange factor activity; histogenesis and organogenesis; signal transduction; zinc ion binding
AA043061	1.68845	DKFZP727G051	26147	DKFZP727G051 protein	DNA binding; regulation of transcription, DNA-dependent
AA004997	1.6817	TRAP100	9862	thyroid hormone receptor-associated protein (100 kDa)	ATP binding; mediator complex; molecular_function unknown; nucleus; regulation of transcription, DNA-dependent
H62557	1.66864	HSPA8	3312	heat shock 70kDa protein 8	ATP binding; heat shock protein activity; intracellular; non-chaperonin molecular chaperone ATPase activity; protein folding
N90938	1.6458	HNRPA2B1	3181	heterogeneous nuclear ribonucleoprotein A2/B1	RNA binding; RNA processing; heterogeneous nuclear ribonucleoprotein; nucleus

N32327	1.62862	CPSF5	11051	cleavage and polyadenylation specific factor 5, 25 kDa	RNA binding; mRNA processing; nucleus
R19119	1.62086				
N90527	1.58095	PIM1	5292	pim-1 oncogene	ATP binding; cAMP-dependent protein kinase activity; cell growth and/or maintenance; cytoplasm; development; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; transferase activity
N20098	1.57982	CD151	977	CD151 antigen	cell adhesion; integral to plasma membrane; membrane fraction
W60305	1.56134				
R84700	1.54625	PKM2	5315	pyruvate kinase, muscle	
H25578	1.54297				
R54918	1.53857	FLJ13912	64785	hypothetical protein FLJ13912	
AA137073	1.51737				
N70221	1.51581	KIAA0500	57237	KIAA0500 protein	
H45355	1.51462				
H64900	1.51382				
H79188	1.50945	ERCC2	2068	excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)	ATP binding; ATP dependent DNA helicase activity; DNA binding; hearing; hydrolase activity; magnesium ion binding; nucleotide-excision repair; nucleus; regulation of transcription, DNA-dependent
AA047172	1.50434	WDR1	9948	WD repeat domain 1	actin binding; cytoskeleton; hearing; protein binding
AA004845	1.50065	KIAA1529	57653	KIAA1529 protein	

AA098865	1.49567	BCL2L10	10017	BCL2-like 10 (apoptosis facilitator)	anti-apoptosis; apoptosis inhibitor activity; caspase activation; integral to membrane; membrane fraction; mitochondrion; oogenesis; protein binding; spermatogenesis
R84451	1.49119				
N23806	1.48899	LOC113386	113386	similar to envelope protein	
W85877	1.48488				
W68333	1.47652		346452	LOC346452	
R87352	1.47143	BCKDHA	593	branched chain keto acid dehydrogenase E1, alpha polypeptide (maple syrup urine disease)	3-methyl-2-oxobutanoate dehydrogenase (lipoamide) activity; 3-methyl-2-oxobutanoate dehydrogenase (lipoamide) complex; alpha-ketoacid dehydrogenase activity; alpha-ketoglutarate dehydrogenase complex (sensu Eukarya); metabolism; mitochondrion
H69656	1.4693	NARF	26502	nuclear prelamin A recognition factor	lamin binding; nuclear lamina
N56656	1.46477	D13S106E	10208	highly charged protein	cysteine-type endopeptidase activity; ubiquitin C-terminal hydrolase activity; ubiquitin-dependent protein catabolism
AA047135	1.46205	RNH	6050	ribonuclease/angiogenin inhibitor	RNA catabolism; ribonuclease inhibitor activity
AA101859	1.45684	ENSA	2029	endosulfine alpha	ion channel inhibitor activity; receptor binding; response to nutrients; transport

N71628	1.45316	SPIB	6689	Spi-B transcription factor (Spi-1/PU.1 related)	RNA polymerase II transcription factor activity; biological_process unknown; cytoplasm; molecular_function unknown; nucleus; regulation of transcription from Pol II promoter; transcription factor activity
AA131782	1.44189				
H83025	1.43173				
W45719	1.42601	PAPA-1	83444	PAP-1 binding protein	
H18298	1.42171				
N39391	1.41993	MGC14799	84296	hypothetical protein MGC14799	
H18495	1.41884				
AA194880	1.41282	DC-UbP	92181	dendritic cell-derived ubiquitin-like protein	
N24337	1.41241	CD44	960	CD44 antigen (homing function and Indian blood group system)	cell adhesion receptor activity; cell-cell adhesion; cell-matrix adhesion; collagen binding; hyaluronic acid binding; integral to plasma membrane; receptor activity
AA148568	1.4095	SNRPB	6628	small nuclear ribonucleoprotein polypeptides B and B1	mRNA splicing; small nuclear ribonucleoprotein; spliceosome complex
AA054271	1.40667	GAPD	2597	glyceraldehyde-3-phosphate dehydrogenase	cytoplasm; glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) activity; glycolysis; oxidoreductase activity
H19297	1.40394	EDIL3	10085	EGF-like repeats and discoidin I-like domains 3	calcium ion binding; cell adhesion; cell adhesion molecule activity; development; integrin binding
W94117	1.40301				
H20520	1.39971				

R85150	1.39948	EPHB6	2051	EphB6	ATP binding; ephrin receptor activity; integral to membrane; protein amino acid phosphorylation; protein tyrosine kinase activity; receptor activity; transmembrane receptor protein tyrosine kinase signaling pathway
AA203242	1.39794	ASB13	79754	ankyrin repeat and SOCS box-containing 13	intracellular signaling cascade
H93330	1.39314	SLC9A3R1	9368	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1	actin cytoskeleton; intracellular signaling cascade; protein complex assembly
W85843	1.39028	LOC253782	253782	hypothetical protein LOC253782	
N43796	1.39023	ATP6V0D1	9114	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d isoform 1	ATP biosynthesis; hydrogen ion transporter activity; hydrogen-translocating V-type ATPase complex; hydrogen-transporting two-sector ATPase activity; hydrolase activity; molecular_function unknown; proton transport
AA057300	1.38962	QDPR	5860	quinoid dihydropteridine reductase	amino acid metabolism; dihydrobiopterin reduction; dihydropteridine reductase activity; electron transporter activity; metabolism; oxidoreductase activity; phenylalanine catabolism; tetrahydrobiopterin biosynthesis

H18900	1.38528	TPI1	7167	triosephosphate isomerase 1	fatty acid biosynthesis; gluconeogenesis; glycolysis; isomerase activity; metabolism; pentose-phosphate shunt; triose-phosphate isomerase activity
H83698	1.38185	MGC10820	84734	hypothetical protein MGC10820	kinesin complex
W89099	1.36991	CYP4F12	66002	cytochrome P450, family 4, subfamily F, polypeptide 12	electron transport; endoplasmic reticulum; membrane; microsome; monooxygenase activity
H69898	1.36373		285465	hypothetical gene supported by AK096576	
H69898	1.36373	FLJ21841	79662	hypothetical protein FLJ21841	
H82992	1.36359	PIGT	51604	phosphatidyl inositol glycan class T	
AA127687	1.36203	MGC3196	79064	hypothetical protein MGC3196	
AA150384	1.35985	NICE-4	9898	NICE-4 protein	
H43455	1.35791	PP2447	80305	hypothetical protein PP2447	
N45640	1.35736	CH25H	9023	cholesterol 25-hydroxylase	catalytic activity; lipid metabolism; membrane fraction; steroid hydroxylase activity
N53883	1.35661	KIAA0276	23142	KIAA0276 protein	
H67193	1.35305	EIF2S3	1968	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	GTPase activity; cytosolic small ribosomal subunit (sensu Eukarya); eukaryotic translation initiation factor 2 complex; translation elongation factor activity; translation initiation factor activity; translational elongation
N47284	1.3508				
R23351	1.34692				
AA059148	1.34334	KIAA1199	57214	KIAA1199 protein	
W46155	1.34043				

W88726	1.3382	MTX1	4580	metaxin 1	integral to membrane
AA213450	1.33654				
W58177	1.33626	HIST2H2AA	8337	histone 2, H2aa	DNA binding; chromosome; chromosome organization and biogenesis (sensu Eukarya); nucleosome; nucleosome assembly; nucleus
N42484	1.33301				
AA019138	1.33299	SLC2A5	6518	solute carrier family 2 (facilitated glucose/fructose transporter), member 5	carbohydrate metabolism; carbohydrate transport; fructose transport; fructose transporter activity; glucose transport; glucose transporter activity; integral to membrane; plasma membrane; sugar porter activity; transporter activity
AA213887	1.32852	FLJ21908	79657	hypothetical protein FLJ21908	
H40607	1.32381				
N95545	1.3227	IL11	3589	interleukin 11	B-cell differentiation; adipocyte differentiation; cell proliferation; cell-cell signaling; cytokine activity; extracellular; interleukin-11 receptor binding; megakaryocyte differentiation; platelet activation; positive regulation of cell proliferation
AA044803	1.32088	FLJ20040	54442	hypothetical protein FLJ20040	membrane; potassium ion transport; protein binding; voltage-gated potassium channel activity; voltage-gated potassium channel complex

AA031859	1.31813	TIMM13	26517	translocase of inner mitochondrial membrane 13 homolog (yeast)	hearing; mitochondrial inner membrane pre-sequence translocase complex; mitochondrial translocation; mitochondrion; protein targeting; protein translocase activity; zinc ion binding
AA142881	1.31641		284354	similar to BC282485_1	
AA210768	1.31532				
H19488	1.31527				
AA203110	1.31081	AIBZIP	148327	androgen-induced basic leucine zipper	DNA binding; nucleus; regulation of transcription, DNA-dependent
H62766	1.30716				
AA002135	1.30424	C2	717	complement component 2	chymotrypsin activity; classical-complement pathway C3/C5 convertase activity; complement activation, classical pathway; complement component C2 complex; hydrolase activity; proteolysis and peptidolysis; trypsin activity
H87311	1.30351	ERCC1	2067	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	DNA repair; embryogenesis and morphogenesis; endodeoxyribonuclease activity; nucleotide-excision repair; nucleus
H84257	1.29776				
H45746	1.29746				
AA127214	1.29622	IGFBP5	3488	insulin-like growth factor binding protein 5	extracellular space; insulin-like growth factor binding; regulation of cell growth; signal transduction
T86338	1.29606				
H26580	1.29372	IGKC	3514	immunoglobulin kappa constant	antigen binding; immune response

H27908	1.29153	TUBB4	10381	tubulin, beta, 4	GTP binding; cytoskeleton; microtubule; microtubule-based movement; structural constituent of cytoskeleton
H26870	1.28945				
H27400	1.28938		286202	LOC286202	
H70974	1.28906				
W47153	1.28838	PTRF	284119	polymerase I and transcript release factor	
N32700	1.28764	RPS3	6188	ribosomal protein S3	RNA binding; cytosolic small ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; structural constituent of ribosome
AA131933	1.28741	ABP1	26	amiloride binding protein 1 (amine oxidase (copper-containing))	amine oxidase (copper-containing) activity; copper ion binding; drug binding; heparin binding; metabolism; oxidoreductase activity; peroxisome
AA046245	1.28676	OSF-2	10631	osteoblast specific factor 2 (fasciclin I-like)	cell adhesion; cell adhesion molecule activity; extracellular matrix; skeletal development
R48060	1.28409	DKFZP564O243	25864	DKFZP564O243 protein	
W45695	1.28288	H2AFZ	3015	H2A histone family, member Z	DNA binding; chromosome; chromosome organization and biogenesis (sensu Eukarya); nucleosome; nucleosome assembly; nucleus
N48735	1.27935				
N24096	1.27886	AKAP12	9590	A kinase (PRKA) anchor protein (gravin) 12	G-protein coupled receptor protein signaling pathway; cytoplasm; protein kinase A anchoring activity; protein targeting; protein transporter activity

H68587	1.27795		340833	LOC340833	
R49189	1.27581	SLC30A6	55676	solute carrier family 30 (zinc transporter), member 6	
AA057286	1.27551	TA-WDRP	134430	T-cell activation WD repeat protein	catalytic activity; metabolism
R49895	1.2755		350854	similar to SNAG1	
W02372	1.27341		284752	LOC284752	
W33064	1.27308	TUBA1	7277	tubulin, alpha 1 (testis specific)	microtubule; structural constituent of cytoskeleton
R88435	1.27112	DPP6	1804	dipeptidylpeptidase 6	catalytic activity; dipeptidyl-peptidase IV activity; dipeptidyl-peptidase activity; integral to membrane; proteolysis and peptidolysis
AA039791	1.27003	ICA1	3382	islet cell autoantigen 1, 69kDa	cytoplasm
W90601	1.26828	HADHA	3030	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	3-hydroxyacyl-CoA dehydrogenase activity; acetyl-CoA C-acetyltransferase activity; fatty acid metabolism; long-chain enoyl-CoA hydratase activity; lyase activity; metabolism; mitochondrion; oxidoreductase activity; short-chain enoyl-CoA hydratase activity
H50204	1.26813	PKM2	5315	pyruvate kinase, muscle	
AA136856	1.26671	TEM7R	84898	tumor endothelial marker 7-related precursor	
AA044889	1.26413				
AA054468	1.26402	MYH11	4629	myosin, heavy polypeptide 11, smooth muscle	ATP binding; actin binding; calmodulin binding; cell growth and/or maintenance; kinesin complex; motor activity; muscle development; muscle myosin; myosin; striated muscle contraction; striated muscle thick filament

R94499	1.26393	GNB5	10681	guanine nucleotide binding protein (G protein), beta 5	
H21137	1.26251				
H52741	1.26191				
W31285	1.26095	TCF3	6929	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	cell growth and/or maintenance; nucleus; regulation of transcription, DNA-dependent; transcription factor activity
N24815	1.25981	UBA52	7311	ubiquitin A-52 residue ribosomal protein fusion product 1	nucleus; protein biosynthesis; protein modification; ribosome; structural constituent of ribosome
AA059335	1.25971				
H70759	1.259				
H86672	1.25743				
R95136	1.25654				
N47105	1.25626	CSPG2	1462	chondroitin sulfate proteoglycan 2 (versican)	calcium ion binding; cell recognition; development; extracellular matrix; heterophilic cell adhesion; hyaluronic acid binding; sugar binding
H61040	1.25569				
H27334	1.25455	DDR1	780	discoidin domain receptor family, member 1	ATP binding; cell adhesion; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; transferase activity; transmembrane receptor protein tyrosine kinase activity; transmembrane receptor protein tyrosine kinase signaling pathway
N31846	1.2541	ACP2	53	acid phosphatase 2, lysosomal	acid phosphatase activity; hydrolase activity; integral to membrane; lysosomal membrane

N72582	1.25336				
R85191	1.25308	FLJ31364	146956	homolog of yeast EME1 endonuclease	
H45010	1.25288	ICAP-1A	9270	integrin cytoplasmic domain-associated protein 1	cell adhesion receptor activity; cell-matrix adhesion; membrane; protein C-terminus binding; protein kinase cascade
N64846	1.25133	KIAA1416	55636	KIAA1416 protein	
AA131302	1.24851				
R88818	1.24836	GSTM1	2944	glutathione S-transferase M1	cytoplasm; glutathione transferase activity; tumor suppressor
W52537	1.24787	PSMA2	5683	proteasome (prosome, macropain) subunit, alpha type, 2	26S proteasome; cytosol; endopeptidase activity; proteasome core complex (sensu Eukarya); proteasome endopeptidase activity; ubiquitin-dependent protein catabolism
R90757	1.24601	RPH3A	22895	likely ortholog of mouse rabphilin 3A	intracellular protein transport; membrane; protein transporter activity; synaptic junction; synaptic vesicle; zinc ion binding
AA026475	1.24268				
N39088	1.24252				
N67453	1.24188	CDKN1A	1026	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	cell cycle arrest; cell cycle regulator; cyclin-dependent protein kinase inhibitor activity; induction of apoptosis by intracellular signals; negative regulation of cell proliferation; nucleus; oncogenesis; protein kinase activity; regulation of CDK activity; regulation of cell cycle; tumor suppressor
N67453	1.24188		286039	similar to hypothetical protein	

H94541	1.24167	C20orf64	112858	chromosome 20 open reading frame 64	ATP binding; nucleus; protein amino acid phosphorylation; protein binding; protein serine/threonine kinase activity; transferase activity
T39206	1.24141				
H26552	1.23804	MGC5395	79026	hypothetical protein MGC5395	intracellular signaling cascade
N50057	1.23646	ORMDL2	29095	ORM1-like 2 (S. cerevisiae)	
N78414	1.23531	LOC144997	144997	hypothetical protein LOC144997	
H69440	1.23469	ANKRD13	88455	ankyrin repeat domain 13	
AA043685	1.23316				
R28329	1.23314	MGC16063	114129	hypothetical protein MGC16063	
AA054115	1.23251				
AA043227	1.23068	CNN3	1266	calponin 3, acidic	actin binding; calmodulin binding; cellular_component unknown; smooth muscle contraction; tropomyosin binding; troponin C binding
AA152194	1.23037	PTP9Q22	138639	protein tyrosine phosphatase PTP9Q22	protein amino acid dephosphorylation; protein tyrosine phosphatase activity; protein tyrosine/serine/threonine phosphatase activity

H71213	1.22872	F2	2147	coagulation factor II (thrombin)	STAT protein nuclear translocation; acute-phase response; apoptosis; blood coagulation; calcium ion binding; caspase activation; chymotrypsin activity; development; extracellular space; hydrolase activity; proteolysis and peptidolysis; regulation of cell cycle; response to wounding; soluble fraction; thrombin activity; trypsin activity; tyrosine phosphorylation of STAT protein
H29730	1.22773				
N91039	1.22672	DDX5	1655	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	ATP binding; ATP dependent helicase activity; RNA helicase activity; cell growth; nucleus
H68805	1.22625				
H93450	1.22599	ZNF347	84671	zinc finger protein 347	DNA binding; nucleus; regulation of transcription, DNA-dependent
H84293	1.22425	SLC12A5	57468	solute carrier family 12, (potassium-chloride transporter) member 5	amino acid transport; amino acid-polyamine transporter activity; cell ion homeostasis; chloride transport; integral to membrane; ion transport; potassium ion transport; potassium:chloride symporter activity; sodium ion transport; symporter activity; transporter activity
T95863	1.22308				
AA115496	1.22285	TRAP25	90390	TRAP/Mediator complex component	
AA058632	1.22273	KIF1B	23095	kinesin family member 1B	

H51834	1.22179	TTC1	7265	tetratricopeptide repeat domain 1	chaperone activity; protein binding; protein folding
T77398	1.22123	AOC3	8639	amine oxidase, copper containing 3 (vascular adhesion protein 1)	amine metabolism; amine oxidase (copper-containing) activity; cell adhesion; cell adhesion molecule activity; copper ion binding; electron transporter activity; inflammatory response; integral to membrane; oxidoreductase activity; plasma membrane
AA151577	1.22066	HPRT1	3251	hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	behavior; cytoplasm; hypoxanthine phosphoribosyltransferase activity; magnesium ion binding; nucleoside metabolism; purine salvage; transferase activity, transferring glycosyl groups
H45972	1.21983				
AA099685	1.2175	PIBF1	10464	progesterone-induced blocking factor 1	
N48160	1.21254	LCMR1	219541	lung cancer metastasis-related protein 1	
AA028111	1.21238	CXorf9	54440	chromosome X open reading frame 9	
H20790	1.21043		348024	similar to TPIP alpha lipid phosphatase	
W33065	1.20973	FLJ12760	339175	hypothetical protein FLJ12760	
R98517	1.20701	HIST1H1C	3006	histone 1, H1c	DNA binding; chromosome; chromosome organization and biogenesis (sensu Eukarya); nucleosome; nucleosome assembly; nucleus
H45472	1.20642				
AA152297	1.20615	PNPO	55163	pyridoxine-5'-phosphate oxidase	pyridoxamine-phosphate oxidase activity; pyridoxine biosynthesis

H83003	1.20601	IGSF1	3547	immunoglobulin superfamily, member 1	cell adhesion; integral to plasma membrane
H51160	1.20446	PPP2R1A	5518	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	protein phosphatase type 2A activity
W37621	1.20397	MEF2B	4207	MADS box transcription enhancer factor 2, polypeptide B (myocyte enhancer factor 2B)	muscle development; nucleus; transcription activating factor; transcription co-activator activity; transcription factor activity; transcription from Pol II promoter
N33086	1.20201				
R89790	1.20005				
H00498	1.19972	PPP2R3A	5523	protein phosphatase 2 (formerly 2A), regulatory subunit B'', alpha	calcium ion binding; protein phosphatase type 2A, intrinsic regulator activity
W25557	1.19882	TRIM28	10155	tripartite motif-containing 28	nucleus; regulation of transcription from Pol II promoter; transcription co-repressor activity; transcription factor activity; zinc ion binding
R47938	1.19757	FLJ32096	148646	hypothetical protein FLJ32096	
H64569	1.19614				
H18190	1.19576	JAK1	3716	Janus kinase 1 (a protein tyrosine kinase)	ATP binding; cytoskeleton; intracellular signaling cascade; protein amino acid phosphorylation; protein tyrosine kinase activity; transferase activity
AA057398	1.19564				
N77703	1.19466	MGC21654	93594	unknown MGC21654 product	
AA039677	1.19306	DKFZP434A0131	54441	DKFZp434A0131 protein	
N71552	1.19282	DKFZp434D1428	84213	hypothetical protein DKFZp434D1428	
R47758	1.19013	LMNB2	84823	lamin B2	S-specific transcription in mitotic cell cycle; lamin filament

H09945	1.18823				
H70359	1.18679				
H68440	1.1866	PIP5K1B	8395	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	
AA204701	1.18363				
R82834	1.18305				
R50905	1.18121	TUBB	7280	tubulin, beta polypeptide	cytoskeleton; structural constituent of cytoskeleton
AA057126	1.17985	KIAA1416	55636	KIAA1416 protein	
N94432	1.17822				
N20665	1.17448	MSI2	124540	musashi homolog 2 (Drosophila)	
AA036801	1.1743	PRDX2	7001	peroxiredoxin 2	antioxidant activity; cytoplasm; electron transporter activity; oxidoreductase activity; response to oxidative stress; thioredoxin peroxidase activity
AA129727	1.17119	RAB5C	5878	RAB5C, member RAS oncogene family	GTP binding; RAB small monomeric GTPase activity; intracellular protein transport; protein transporter activity; small GTPase mediated signal transduction
H61812	1.16962	CDK4	1019	cyclin-dependent kinase 4	G1/S transition of mitotic cell cycle; cell proliferation; cyclin-dependent protein kinase activity; oncogenesis; regulation of cell cycle
N28915	1.16928	CERK	64781	ceramide kinase	
R23374	1.16888	FLJ10462	55711	hypothetical protein FLJ10462	
H63763	1.16855				
W85995	1.1684				
H45128	1.16752	IGHG3	3502	immunoglobulin heavy constant gamma 3 (G3m marker)	antigen binding; immune response; membrane fraction
H67696	1.16713				
R01530	1.16687				

N31469	1.16319	NCKAP1	10787	NCK-associated protein 1	apoptosis; central nervous system development; integral to membrane
AA031564	1.16281	LOC113444	113444	hypothetical protein BC011880	
N47654	1.16033	KIAA0140	9679	KIAA0140 gene product	
R81035	1.15962	EIF5A	1984	eukaryotic translation initiation factor 5A	
H84008	1.15948				
R72577	1.15882	FLJ11753	79712	hypothetical protein FLJ11753	
AA054170	1.15736				
AA059274	1.15565	KIAA1594	57695	KIAA1594 protein	
H93017	1.15546	ECH1	1891	enoyl Coenzyme A hydratase 1, peroxisomal	energy pathways; enoyl-CoA hydratase activity; fatty acid beta-oxidation; fatty acid metabolism; isomerase activity; mitochondrion; peroxisome
H95467	1.1545	MIDORI	57538	likely ortholog of mouse myocytic induction/differentiation originator	ATP binding; kinase activity; protein amino acid phosphorylation; protein serine/threonine kinase activity
AA114905	1.15449	SPAG7	9552	sperm associated antigen 7	nucleic acid binding
R21970	1.15446	GTF2H2	2966	general transcription factor IIH, polypeptide 2, 44kDa	DNA repair; nucleus; regulation of transcription, DNA-dependent
AA033685	1.15394				
AA121514	1.1534	ZNF197	10168	zinc finger protein 197	transcription factor activity
R53840	1.15265	RABGEF1	27342	RAB guanine nucleotide exchange factor (GEF) 1	DNA binding; zinc ion binding
R21702	1.15133				

H84617	1.15113	PCAF	8850	p300/CBP-associated factor	N-acetyltransferase activity; cell cycle; cell cycle arrest; chromatin modeling; histone deacetylase activity; negative regulation of cell proliferation; nucleus; protein amino acid acetylation; regulation of transcription, DNA-dependent; transcription cofactor activity; transferase activity
H59810	1.15093	CLU	1191	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	apoptosis; cell death; complement activation, classical pathway; fertilization (sensu Animalia); lipid metabolism
N31625	1.14819	KIAA1909	153478	KIAA1909 protein	
AA135646	1.14651	hIAN6	155038	human immune associated nucleotide 6	
N30177	1.14608	LOC285958	285958	hypothetical protein LOC285958	
T87888	1.14578	KIAA1046	22867	KIAA1046 protein	
AA045281	1.1453	PAG	55824	phosphoprotein associated with glycosphingolipid-enriched microdomains	antimicrobial humoral response (sensu Invertebrata); integral to plasma membrane; signal transduction; transmembrane receptor protein tyrosine kinase adaptor protein activity
AA046610	1.14455				
H62770	1.13654				
N30471	1.13607	DKFZp586I1420	222161	hypothetical protein DKFZp586I1420	
W07300	1.13597	AP1G1	164	adaptor-related protein complex 1, gamma 1 subunit	Golgi apparatus; clathrin adaptor; coated pit; endocytosis; intracellular protein transport; protein transporter activity
AA069532	1.13508				

AA203751	1.13496	KIAA1956	147686	KIAA1956 protein	DNA binding; nucleus; regulation of transcription, DNA-dependent
H84844	1.13238				
R85333	1.13207				
R26844	1.131				
R47859	1.13045	NPR1	4881	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	ATP binding; cGMP biosynthesis; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; peptide receptor activity, G-protein coupled; protein amino acid phosphorylation; protein kinase activity; receptor activity; receptor guanylate cyclase activity; regulation of blood pressure
H66198	1.12923		343171	similar to seven transmembrane helix receptor	
N92573	1.12567	MVD	4597	mevalonate (diphospho) decarboxylase	cholesterol biosynthesis; diphosphomevalonate decarboxylase activity; isoprenoid biosynthesis; lyase activity
H50914	1.12561		284665	hypothetical gene supported by BC023596	
N56889	1.12525	SOS2	6655	son of sevenless homolog 2 (Drosophila)	cellular_component unknown; guanyl-nucleotide exchange factor activity; small GTPase mediated signal transduction
AA099647	1.12465	TSPAN-2	10100	tetraspan 2	cell adhesion; cell motility; cell proliferation; integral to membrane; mystery cell fate differentiation (sensu Drosophila)
H60488	1.12462	H326	50717	H326	

H27352	1.12415	HRAS	3265	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	GTPase activity; cell motility; cell shape and cell size control; cell surface receptor linked signal transduction; chemotaxis; cytoplasm; histogenesis and organogenesis; peripheral plasma membrane protein; plasma membrane; regulation of cell cycle; signal transduction
AA149333	1.1237	ACTN1	87	actinin, alpha 1	actin binding; actin cytoskeleton; calcium ion binding; structural constituent of cytoskeleton
AA210872	1.12192	DKFZp667E0512	202025	hypothetical protein DKFZp667E0512	
R90824	1.11871	TMEM10	93377	transmembrane protein 10	integral to membrane
W52472	1.11848	PCDH1	5097	protocadherin 1 (cadherin-like 1)	
H71358	1.11817				
R41363	1.11746				
R28090	1.11676	KIAA1495	57631	KIAA1495 protein	
N32669	1.11488	RBT1	29946	RPA-binding trans-activator	
R71629	1.11417	LST1	7940	leukocyte specific transcript 1	cellular defense response; defense/immunity protein activity; immune response; integral to plasma membrane
AA136159	1.11406	MGST1	4257	microsomal glutathione S-transferase 1	glutathione transferase activity; membrane; microsome; mitochondrion; transferase activity
H14332	1.11314				
H65775	1.11187				
N45213	1.11181		147808	similar to zinc finger protein Zec	
N22392	1.11168	CLDN11	5010	claudin 11 (oligodendrocyte transmembrane	integral to membrane; structural molecule activity; tight junction

				protein)	
AA054473	1.11148	GOSR2	9570	golgi SNAP receptor complex member 2	ER to Golgi transport; Golgi apparatus; integral to membrane; intracellular protein transport; kinesin complex; membrane fusion; protein transporter activity; v-SNARE activity
H30081	1.10991				
AA054300	1.10989	KLHL4	56062	kelch-like 4 (Drosophila)	actin binding; actin cytoskeleton organization and biogenesis; cytoskeleton; protein binding
W02842	1.10882	TBX2	6909	T-box 2	development; nucleus; regulation of transcription, DNA-dependent; transcription factor activity
H59454	1.1079				
H46133	1.10788	BAI2	576	brain-specific angiogenesis inhibitor 2	G-protein coupled receptor activity; integral to membrane; neuropeptide signaling pathway
AA021582	1.10723	GFAP	2670	glial fibrillary acidic protein	intermediate filament; structural constituent of cytoskeleton
H59405	1.10629	FLJ10298	54682	hypothetical protein FLJ10298	
R28033	1.10612	PTPN18	26469	protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	non-membrane spanning protein tyrosine phosphatase activity; protein amino acid dephosphorylation
AA142943	1.10577	DOK1	1796	docking protein 1, 62kDa (downstream of tyrosine kinase 1)	cell surface receptor linked signal transduction; insulin receptor binding; protein binding; transmembrane receptor protein tyrosine kinase signaling pathway

W04610	1.10503	H3F3A	3020	H3 histone, family 3A	
AA045373	1.10484	TCEAL1	9338	transcription elongation factor A (SII)-like 1	RNA polymerase II transcription factor activity; negative regulation of transcription from Pol II promoter; nucleus; regulation of transcription, DNA-dependent; transcription factor activity; translation elongation factor activity
N45505	1.10213	VAV1	7409	vav 1 oncogene	cell growth and/or maintenance; diacylglycerol binding; guanyl-nucleotide exchange factor activity; intracellular signaling cascade; nucleus; transcription factor activity
N45013	1.10126				
R22402	1.10059				
H86198	1.10011				
AA034916	1.09997	FLJ11457	79809	hypothetical protein FLJ11457	
N90061	1.09992	STE	6783	sulfotransferase, estrogen-preferring	estrone sulfotransferase activity; steroid binding; steroid metabolism; transferase activity
H52939	1.09857				
H16193	1.09761				
AA035066	1.0976	MGC4268	83607	hypothetical protein MGC4268	
AA040852	1.09709	KIAA1321	57532	KIAA1321 protein	
H88577	1.09513	HNRPH1	3187	heterogeneous nuclear ribonucleoprotein H1 (H)	RNA binding; RNA processing; heterogeneous nuclear ribonucleoprotein; nucleus; poly(U) binding; ribonucleoprotein complex

N90836	1.09501	FMR1	2332	fragile X mental retardation 1	mRNA binding; nucleoplasm; polysome; soluble fraction
AA044181	1.09376	ENAH	55740	enabled homolog (Drosophila)	
H23933	1.09286				
AA131391	1.09165	TRIM29	23650	tripartite motif-containing 29	transcription factor activity; transcription from Pol II promoter
R85044	1.09161	SMPD1	6609	sphingomyelin phosphodiesterase 1, acid lysosomal (acid sphingomyelinase)	carbohydrate metabolism; hydrolase activity, acting on glycosyl bonds; lysosome; neurogenesis; signal transduction; sphingomyelin metabolism; sphingomyelin phosphodiesterase activity
N22938	1.09154	SAA4	6291	serum amyloid A4, constitutive	acute-phase response; acute-phase response protein activity; extracellular; lipid transporter activity
R88711	1.09131				
R85232	1.09047	ACCN2	41	amiloride-sensitive cation channel 2, neuronal	amiloride-sensitive sodium channel activity; integral to membrane; ion channel activity; ion transport; sodium ion transport
H71112	1.0887	MCM2	4171	MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)	ATP binding; DNA binding; DNA dependent ATPase activity; DNA replication; DNA replication initiation; cell cycle; chromatin; nucleus; regulation of transcription, DNA-dependent

T54547	1.08811	DF	1675	D component of complement (adipsin)	chymotrypsin activity; complement activation, alternative pathway; complement factor D activity; hydrolase activity; proteolysis and peptidolysis; trypsin activity
W40304	1.08804	API5	8539	apoptosis inhibitor 5	anti-apoptosis; apoptosis inhibitor activity
AA041264	1.08588	ATP2B1	490	ATPase, Ca++ transporting, plasma membrane 1	ATP binding; calcium ion transport; calcium-transporting ATPase activity; calmodulin binding; cation transport; hydrolase activity; integral to plasma membrane; magnesium ion binding; metabolism; transport
H38321	1.08519	FLJ14360	84861	hypothetical protein FLJ14360	protein binding
N81000	1.08328				
H52253	1.08181	IGHG3	3502	immunoglobulin heavy constant gamma 3 (G3m marker)	antigen binding; immune response; membrane fraction
H40662	1.08154	KNS2	3831	kinesin 2 60/70kDa	kinesin complex; microtubule motor activity; microtubule-based process
H68885	1.08128	TSSC3	7262	tumor suppressing subtransferable candidate 3	apoptosis; imprinting
H59563	1.081	CASP10	843	caspase 10, apoptosis-related cysteine protease	
N74428	1.08013				
R46282	1.07975				
AA101839	1.07876				
N93133	1.07856				
N40017	1.07723	MRPL24	79590	mitochondrial ribosomal protein L24	intracellular; protein biosynthesis; ribosome; structural constituent of ribosome

AA037284	1.07698	APRT	353	adenine phosphoribosyltransferase	adenine phosphoribosyltransferase activity; adenine salvage pathway; nucleoside metabolism; transferase activity, transferring glycosyl groups
AA011556	1.07686				
T66831	1.07643				
AA207094	1.07521				
W37783	1.07349	RRAS2	22800	related RAS viral (r-ras) oncogene homolog 2	GTP binding; RAS small monomeric GTPase activity; cell growth and/or maintenance; endoplasmic reticulum; plasma membrane; small GTPase mediated signal transduction
H12075	1.07259	FLJ37953	129450	hypothetical protein FLJ37953	
H69011	1.07177	SKIL	6498	SKI-like	cell differentiation; cell growth and/or maintenance; molecular_function unknown; nucleus
W90661	1.07093				
R96672	1.07087	CYP2D6	1565	cytochrome P450, family 2, subfamily D, polypeptide 6	cytochrome P450 activity
R87923	1.07057	RPIP8	10900	RaP2 interacting protein 8	small GTPase mediated signal transduction; small GTPase regulatory/interacting protein activity
N34901	1.06882	GALNT7	117248	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7	

AA057293	1.06798	PPIC	5480	peptidylprolyl isomerase C (cyclophilin C)	FK506-sensitive peptidyl-prolyl cis-trans isomerase; antimicrobial humoral response (sensu Invertebrata); chaperone activity; cyclophilin; cyclophilin-type peptidy-prolyl cis-trans isomerase activity; cyclosporin A binding; cytoplasm; isomerase activity; protein folding; signal transduction
AA147500	1.06795		282963	hypothetical gene supported by AL833529; AK021993	
H45213	1.066				
H85193	1.06535				
N41763	1.06085				
AA147534	1.06047				
H91962	1.06007				
AA213442	1.05913				
AA036758	1.05844	S100A4	6275	S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)	calcium ion binding; invasive growth
R88082	1.0574				
W42634	1.05675	FAP	2191	fibroblast activation protein, alpha	dipeptidyl-peptidase IV activity; integral to membrane; prolyl oligopeptidase activity; proteolysis and peptidolysis
R54729	1.05414				
H41751	1.05392	GTF2F1	2962	general transcription factor IIF, polypeptide 1, 74kDa	DNA binding; general RNA polymerase II transcription factor activity; nucleus; protein binding; regulation of transcription, DNA-dependent; transcription co-activator activity; transcription factor TFIIF complex; transcription initiation from Pol II promoter

AA204664	1.05298	SMC1L2	27127	SMC1 structural maintenance of chromosomes 1-like 2 (yeast)	ATP binding; ATP-binding cassette (ABC) transporter activity; cell cycle; chromosome segregation; kinesin complex; meiosis; membrane; nucleus; transport
N30528	1.05281	PPARD	5467	peroxisome proliferative activated receptor, delta	
N70531	1.05242	STMN1	3925	stathmin 1/oncoprotein 18	cell growth and/or maintenance; cytosol; intracellular signaling cascade; kinesin complex; microtubule-based process; signal transducer activity
R66261	1.05162	PRG2	5553	proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein)	cytoplasm; extracellular; heparin binding; heterophilic cell adhesion; immune response; inflammatory response; lectin; sugar binding; toxin activity; xenobiotic metabolism
N78926	1.0507	MYL6	4637	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	muscle myosin; non-muscle myosin; structural constituent of muscle
R48615	1.04859	C14orf21	161424	chromosome 14 open reading frame 21	RNA binding
H49225	1.04854				
R51354	1.04815	CNTNAP2	26047	contactin associated protein-like 2	
AA063424	1.04652	C6orf80	25901	chromosome 6 open reading frame 80	
N63012	1.04631				
W49770	1.04582	MORF4L2	9643	mortality factor 4 like 2	molecular_function unknown; nucleus; regulation of cell growth

R53559	1.04558	NME1	4830	non-metastatic cells 1, protein (NM23A) expressed in	ATP binding; CTP biosynthesis; GTP biosynthesis; UTP biosynthesis; kinase activity; negative regulation of cell cycle; negative regulation of cell proliferation; nucleoside triphosphate biosynthesis; nucleoside-diphosphate kinase activity; nucleus; transferase activity
AA115689	1.04489				
AA028109	1.04465	RAB23	51715	RAB23, member RAS oncogene family	GTP binding; RAB small monomeric GTPase activity; intracellular protein transport; protein transporter activity; small GTPase mediated signal transduction
N33745	1.04362	KIAA0841	23354	KIAA0841 protein	nucleic acid binding
W04822	1.04293				
H02088	1.04178	RBAF600	23352	retinoblastoma-associated factor 600	
H30357	1.04171	ATP4A	495	ATPase, H+/K+ exchanging, alpha polypeptide	ATP binding; hydrogen/potassium-exchanging ATPase activity; hydrolase activity; integral to plasma membrane; magnesium ion binding; metabolism; potassium ion transport; proton transport; transport
N39691	1.04084	CFL1	1072	cofilin 1 (non-muscle)	Rho protein signal transduction; actin cytoskeleton organization and biogenesis; actin modulating activity; cytoskeleton; nucleus
H27034	1.04005	IGKC	3514	immunoglobulin kappa constant	antigen binding; immune response
AA203153	1.03836				

AA036635	1.03821	AKAP10	11216	A kinase (PRKA) anchor protein 10	mitochondrion; protein binding; protein localization; signal transducer activity; signal transduction
W52156	1.03787	OXTR	5021	oxytocin receptor	G-protein signaling, coupled to IP3 second messenger (phospholipase C activating); endosome; integral to plasma membrane; lactation; muscle contraction; oxytocin receptor activity; pregnancy; rhodopsin-like receptor activity; vasopressin receptor activity
T77422	1.0375				
W01319	1.03748	BHC80	51317	BRAF35/HDAC2 complex (80 kDa)	
T84788	1.03644				
N55079	1.0356	F10	2159	coagulation factor X	blood coagulation; blood coagulation factor X activity; calcium ion binding; chymotrypsin activity; extracellular; hydrolase activity; proteolysis and peptidolysis; trypsin activity
AA131450	1.03424				
R20373	1.03415	TMP21	10972	transmembrane trafficking protein	ER to Golgi transport; Golgi apparatus; integral to plasma membrane; intracellular protein transport; membrane fraction; microsome; protein carrier activity; protein transporter activity
R47945	1.03168	CCT5	22948	chaperonin containing TCP1, subunit 5 (epsilon)	ATP binding; chaperone activity

H38879	1.02995	PSPH	5723	phosphoserine phosphatase	hydrolase activity; magnesium ion binding; metabolism; phosphoserine phosphatase activity; serine biosynthesis
R44307	1.02859	PPP1R9B	84687	protein phosphatase 1, regulatory subunit 9B, spinophilin	intracellular signaling cascade; membrane; transport; transporter activity
W87556	1.02814				
N80976	1.02773	LOC51252	51252	hypothetical protein LOC51252	
AA059076	1.02713	MTMR9	66036	myotubularin related protein 9	
H86858	1.02685				
W89025	1.02603				
N91128	1.02596	SFTPB	6439	surfactant, pulmonary-associated protein B	extracellular space; histogenesis and organogenesis; lysosome; respiratory gaseous exchange; sphingolipid metabolism; surfactant activity
H04530	1.02471	ECHS1	1892	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	energy pathways; fatty acid beta-oxidation; fatty acid metabolism; long-chain enoyl-CoA hydratase activity; lyase activity; mitochondrion; short-chain enoyl-CoA hydratase activity
N73980	1.02454	KRT8	3856	keratin 8	cytoskeleton organization and biogenesis; intermediate filament; kinesin complex; phosphorylation; structural molecule activity
H58631	1.02371				
W42638	1.02059	STAM2	10254	signal transducing adaptor molecule (SH3 domain and ITAM motif) 2	intracellular protein transport
R39421	1.02039	PIGM	93183	phosphatidylinositol glycan, class M	transferase activity

R89056	1.02026	LAMP1	3916	lysosomal-associated membrane protein 1	integral to plasma membrane; lysosome; membrane fraction
H44888	1.01975				
W88660	1.01804	PDIP38	26073	polymerase delta interacting protein 38	
AA056998	1.01557				
N25523	1.01481	HSPE1	3336	heat shock 10kDa protein 1 (chaperonin 10)	co-chaperonin activity; heat shock protein activity; mitochondrion; protein folding
R82691	1.01433		348700	similar to RAN-binding protein 2-like 1 isoform 1; sperm membrane protein BS-63; RAN-binding protein 2-like 1	
W24523	1.01417	MGC20262	138311	hypothetical protein MGC20262	
AA054948	1.01407				
AA069448	1.01373				
N36272	1.01165				
H59595	1.01126				
AA043638	1.00939				
AA127098	1.00921				
W19744	1.00892				
N57249	1.00664	PSMB5	5693	proteasome (prosome, macropain) subunit, beta type, 5	26S proteasome; cytosol; endopeptidase activity; proteasome core complex (sensu Eukarya); proteasome endopeptidase activity; ubiquitin-dependent protein catabolism
R08339	1.00641				
R48610	1.00556	TTC7	57217	tetratricopeptide repeat domain 7	
AA029012	1.00498	SMA5	11042	SMA5	biological_process unknown; carbohydrate metabolism; cellular_component unknown; hydrolase activity, hydrolyzing O-glycosyl compounds; molecular_function unknown

AA036787	1.00455	NCK2	8440	NCK adaptor protein 2	intracellular signaling cascade; negative regulation of cell proliferation; regulation of EGF receptor activity
R07186	1.00438				
AA002041	1.00431	ZNF262	9202	zinc finger protein 262	DNA binding; development; extracellular; hormone activity
N78467	1.00392	PWP1	11137	nuclear phosphoprotein similar to S. cerevisiae PWP1	nucleus; transcription
W78129	1.00383	FGG	2266	fibrinogen, gamma polypeptide	blood coagulation; fibrinogen; fibrinogen gamma chain; positive regulation of cell proliferation; regulation of blood pressure
H14840	1.00372	MGC2668	81605	hypothetical protein MGC2668	
H68441	1.00241	FLJ14054	79614	hypothetical protein FLJ14054	
R74161	1.00161	PYGL	5836	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	carbohydrate metabolism; glycogen metabolism; glycogen phosphorylase activity; transferase activity, transferring glycosyl groups
R77028	1.00093	LTBR	4055	lymphotoxin beta receptor (TNFR superfamily, member 3)	apoptosis; immune response; integral to membrane; signal transduction; transmembrane receptor activity
R50087	1.00091	GREB1	9687	GREB1 protein	
AA131884	1.00077	LOC51057	51057	hypothetical protein LOC51057	
R07617	-1.0006	ABCD4	5826	ATP-binding cassette, sub-family D (ALD), member 4	ATP binding; ATP-binding cassette (ABC) transporter activity; integral to membrane; membrane fraction; nucleotide binding; peroxisomal membrane; transport; transporter activity

T89328	-1.0012	PPP1R12A	4659	protein phosphatase 1, regulatory (inhibitor) subunit 12A	actin cytoskeleton; regulation of muscle contraction; signal transducer activity
W52903	-1.0018	HSPC177	51510	hypothetical protein HSPC177	molecular_function unknown
R00555	-1.007				
R80587	-1.0082	PPP2R2A	5520	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	protein amino acid dephosphorylation; protein phosphatase type 2A complex; protein phosphatase type 2A, intrinsic regulator activity; signal transduction
T86348	-1.0112	GATM	2628	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	creatine biosynthesis; cytosol; glycine amidinotransferase activity; mitochondrion; transferase activity
R46353	-1.0127	AEBP1	165	AE binding protein 1	carboxypeptidase A activity; carboxypeptidase activity; cell adhesion; cytoplasm; muscle development; proteolysis and peptidolysis; skeletal development; transcription factor activity
R26717	-1.0129	LOC134147	134147	hypothetical protein BC001573	hydrolase activity
R75921	-1.0145	SDBCAG84	51614	serologically defined breast cancer antigen 84	
W04539	-1.0151	KIAA0295	23060	KIAA0295 protein	
AA129569	-1.0162	SLC35E2	9906	solute carrier family 35, member E2	
N69900	-1.0163	NIN283	84937	nerve injury gene 283	
T81574	-1.0203				
N79754	-1.0213	ELAC1	55520	elaC homolog 1 (E. coli)	
H51151	-1.0224	GABARAPL1	23710	GABA(A) receptor-associated protein like 1	receptor activity

AA043506	-1.0225	CEBPD	1052	CCAAT/enhancer binding protein (C/EBP), delta	DNA binding; nucleus; regulation of transcription, DNA-dependent; transcription from Pol II promoter
R53445	-1.0227				
T84038	-1.0257	POU2F1	5451	POU domain, class 2, transcription factor 1	nucleus; regulation of transcription, DNA-dependent; transcription factor activity
R20409	-1.0259				
R20620	-1.0272	LOC64174	64174	putative dipeptidase	
AA148455	-1.0278	UBE2V2	7336	ubiquitin-conjugating enzyme E2 variant 2	cell proliferation; ligase activity; protein modification; protein polyubiquitination; regulation of DNA repair; regulation of cell cycle; ubiquitin conjugating enzyme activity; ubiquitin cycle; ubiquitin-protein ligase activity
AA036809	-1.0279				
H64812	-1.0289	RPS28	6234	ribosomal protein S28	RNA binding; cytosolic small ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; ribosome; structural constituent of ribosome
R06463	-1.029	TRAP1	10131	heat shock protein 75	ATP binding; biological_process unknown; cellular_component unknown; chaperone activity; mitochondrion; tumor necrosis factor receptor binding
N74391	-1.0328				
N63587	-1.0384	RPL24	6152	ribosomal protein L24	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; ribosome; structural constituent of ribosome

R12744	-1.0392	SPARC	6678	secreted protein, acidic, cysteine-rich (osteonectin)	basement membrane; calcium ion binding; collagen binding; ossification
N75422	-1.0417	FLJ22662	79887	hypothetical protein FLJ22662	
H06405	-1.0465	PCTK1	5127	PCTAIRE protein kinase 1	protein amino acid phosphorylation; protein kinase activity; protein serine/threonine kinase activity; regulation of cell cycle
R55277	-1.0469				
W40409	-1.0485	KARS	3735	lysyl-tRNA synthetase	
T99304	-1.0525	ZNF297B	23099	zinc finger protein 297B	DNA binding; nucleus; protein binding; regulation of transcription, DNA-dependent
AA056130	-1.0558	PTK7	5754	PTK7 protein tyrosine kinase 7	plasma membrane; proteoglycan integral to plasma membrane; receptor activity; signal transduction; transmembrane receptor protein tyrosine kinase activity
R55995	-1.0573				
H08370	-1.0653	A2BP1	54715	ataxin 2-binding protein 1	Golgi apparatus; RNA binding
N39407	-1.0671	KIF21A	55605	kinesin family member 21A	
AA101827	-1.0723				
W32710	-1.0724		348153	similar to nuclear pore complex interacting protein	
W38749	-1.0733	MAL2	114569	mal, T-cell differentiation protein 2	integral to membrane
N73898	-1.0798	EIF2B1	1967	eukaryotic translation initiation factor 2B, subunit 1 alpha, 26kDa	GTP binding; eukaryotic translation initiation factor 2B complex; guanyl-nucleotide exchange factor activity; translation initiation factor activity; translational initiation
AA031950	-1.0821				

R00507	-1.0827	FLJ33084	149483	hypothetical protein FLJ33084	
T93322	-1.0831				
N75518	-1.0849	TPR	7175	translocated promoter region (to activated MET oncogene)	cytoplasm; kinesin complex; nuclear pore; nucleus; protein-nucleus import; transport
T69714	-1.0878	POLB	5423	polymerase (DNA directed), beta	DNA binding; DNA dependent DNA replication; DNA repair; alpha DNA polymerase activity; beta DNA polymerase activity; delta DNA polymerase activity; deoxycytidyl transferase activity, template dependent; epsilon DNA polymerase activity; eta DNA polymerase activity; gamma DNA-directed DNA polymerase activity; iota DNA polymerase activity; kappa DNA polymerase activity; lambda DNA polymerase activity; mu DNA polymerase activity; nu DNA polymerase activity; nucleus; sigma DNA polymerase activity; theta DNA polymerase activity; transferase activity; zeta DNA polymerase activity
N66814	-1.0891	TRIM32	22954	tripartite motif-containing 32	nucleus; transcription co-activator activity; zinc ion binding
AA151307	-1.0911	GNB2	2783	guanine nucleotide binding protein (G protein), beta polypeptide 2	G-protein coupled receptor protein signaling pathway; heterotrimeric G-protein GTPase activity; heterotrimeric G-protein complex; signal transducer activity; signal transduction

N54788	-1.0942	CYBRD1	79901	cytochrome b reducatse 1	electron transport; integral to membrane
R34626	-1.1005				
R75598	-1.1008	NBL1	4681	neuroblastoma, suppression of tumorigenicity 1	negative regulation of cell cycle
H00122	-1.103	ADCY1	107	adenylate cyclase 1 (brain)	
H09869	-1.1039	GNAI2	2771	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	G-protein coupled receptor protein signaling pathway; GTP binding; heterotrimeric G-protein GTPase activity; negative regulation of adenylate cyclase activity; response to nutrients; signal transducer activity; signal transduction
AA040090	-1.1059	LOC92979	92979	hypothetical protein BC009489	
R35596	-1.1104	SCHIP1	29970	schwannomin interacting protein 1	cytoplasm
AA129598	-1.112	NCE2	140739	NEDD8-conjugating enzyme	ligase activity; protein modification; ubiquitin conjugating enzyme activity; ubiquitin cycle; ubiquitin-protein ligase activity
W37491	-1.1126	BLVRB	645	biliverdin reductase B (flavin reductase (NADPH))	biliverdin reductase activity; flavin reductase activity; oxidoreductase activity
W05611	-1.1185				
N69433	-1.1188				
H94329	-1.1203	DMD	1756	dystrophin (muscular dystrophy, Duchenne and Becker types)	cell shape and cell size control; cytoskeleton; muscle contraction; muscle development; peripheral plasma membrane protein; structural constituent of cytoskeleton
R85505	-1.1218				
R54798	-1.1241	FLJ31810	158038	hypothetical protein FLJ31810	

AA121547	-1.1282	IMPDH2	3615	IMP (inosine monophosphate) dehydrogenase 2	GMP biosynthesis; IMP dehydrogenase activity; oxidoreductase activity; purine nucleotide biosynthesis
AA031958	-1.1321				
T98046	-1.1324	PFDN4	5203	prefoldin 4	chaperonin-mediated tubulin folding; co-chaperone activity; cytosol; protein binding; protein folding; tubulin-specific chaperone activity
H09331	-1.1324	DKFZp434D177	84224	hypothetical protein DKFZp434D177	
N89807	-1.1348	PDGFC	56034	platelet derived growth factor C	
AA001906	-1.1363	TRAP240	9969	thyroid hormone receptor-associated protein, 240 kDa subunit	nucleus; receptor activity; regulation of transcription, DNA-dependent
W24393	-1.1373	STX10	8677	syntaxin 10	Golgi membrane; integral to membrane; intracellular protein transport; kinesin complex; protein transporter activity
R28325	-1.1527	MGC10500	83719	hypothetical protein MGC10500	
T94861	-1.1564	PPP1R15B	84919	protein phosphatase 1, regulatory (inhibitor) subunit 15B	
R40485	-1.159	MMP16	4325	matrix metalloproteinase 16 (membrane-inserted)	collagen catabolism; enzyme activator activity; extracellular matrix; hydrolase activity; integral to plasma membrane; metalloendopeptidase activity; zinc ion binding
T97765	-1.1651				

N91912	-1.1656	PLA2G12	81579	phospholipase A2, group XII	biological_process unknown; calcium ion binding; calcium-dependent cytosolic phospholipase A2 activity; calcium-dependent secreted phospholipase A2 activity; calcium-independent cytosolic phospholipase A2 activity; cellular_component unknown; hydrolase activity; lipid catabolism
H93058	-1.1701		285707	hypothetical gene supported by BC035411	
R43193	-1.1734	FLJ11749	79643	hypothetical protein FLJ11749	molecular_function unknown
R41846	-1.1771				
H28801	-1.1838	AD158	84230	hypothetical protein AD158	
R56495	-1.1919	IKBKG	8517	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	NIK-I-kappaB/NF-kappaB cascade; immune response; induction of apoptosis; kinesin complex; nucleus; regulation of transcription, DNA-dependent; signal transducer activity
W79682	-1.1937	LOC51035	51035	ORF	
W19514	-1.1961	LOC158427	158427	PP4189	
R80802	-1.1987	KDELR1	10945	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	KDEL sequence binding; endoplasmic reticulum; integral to membrane; intracellular protein transport; membrane fraction; protein transporter activity; receptor activity
N71104	-1.1998		283520	hypothetical gene supported by AK095358	

W52798	-1.2049	EMD	2010	emerin (Emery-Dreifuss muscular dystrophy)	integral to membrane; muscle contraction; muscle development; nonselective vesicle transport; nuclear membrane
T98139	-1.2051	HLA-B	3106	major histocompatibility complex, class I, B	MHC class I receptor activity; antigen presentation, endogenous antigen; antigen processing, endogenous antigen via MHC class I; immune response; integral to plasma membrane
T85060	-1.2115				
R40307	-1.2133				
AA149232	-1.2165	SREBF2	6721	sterol regulatory element binding transcription factor 2	DNA binding; Golgi apparatus; RNA polymerase II transcription factor activity; cholesterol metabolism; endoplasmic reticulum; integral to membrane; lipid metabolism; nucleus; regulation of transcription from Pol II promoter
H99444	-1.2218	DKFZP564D172	83989	hypothetical protein DKFZp564D172	
N74415	-1.2274				
R75796	-1.232	PABPN1	8106	poly(A) binding protein, nuclear 1	
N71043	-1.2357	SRPR	6734	signal recognition particle receptor ('docking protein')	GTP binding; cotranslational membrane targeting; integral to membrane; nucleotide binding; protein targeting; receptor activity; signal recognition particle; signal recognition particle binding; signal recognition particle receptor complex
N/A2	-1.239				

R34387	-1.2463	FAAH	2166	fatty acid amide hydrolase	amidase activity; fatty acid metabolism; insoluble fraction; membrane fraction; receptor binding
AA195088	-1.2496				
AA010141	-1.2497	SERPINH1	871	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	heat shock response
N69956	-1.2507	LOC283904	283904	hypothetical protein LOC283904	
N77925	-1.2561	NDUFA1	4694	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa	NADH dehydrogenase (ubiquinone) activity; NADH dehydrogenase activity; energy pathways; membrane fraction; mitochondrion; oxidoreductase activity
T94447	-1.2644	CTXL	23584	cortical thymocyte receptor (X. laevis CTX) like	antigen binding; integral to plasma membrane; membrane fraction
AA046449	-1.2701	ARF1	375	ADP-ribosylation factor 1	Golgi apparatus; intracellular protein transport; plasma membrane; protein transporter activity; receptor signaling protein activity; small GTPase mediated signal transduction; small monomeric GTPase activity
AA057729	-1.2746	FLJ13236	79962	hypothetical protein FLJ13236	
H63653	-1.2777	HLA-B	3106	major histocompatibility complex, class I, B	MHC class I receptor activity; antigen presentation, endogenous antigen; antigen processing, endogenous antigen via MHC class I; immune response; integral to plasma membrane

R07336	-1.279	KCNH2	3757	potassium voltage-gated channel, subfamily H (eag-related), member 2	cation transport; delayed rectifier potassium channel activity; hearing; integral to membrane; membrane fraction; muscle contraction; potassium ion transport; regulation of heart rate; two-component sensor molecule activity; two-component signal transduction system (phosphorelay); voltage-gated potassium channel complex
N53657	-1.3195				
R13346	-1.3199	ENT4	222962	equilibrative nucleoside transporter 4	
AA055938	-1.3223	LAMA4	3910	laminin, alpha 4	basal lamina; extracellular matrix glycoprotein
R38827	-1.3263	CACNA2D2	9254	calcium channel, voltage-dependent, alpha 2/delta subunit 2	membrane
AA045613	-1.3668	HSD11B1	3290	hydroxysteroid (11-beta) dehydrogenase 1	11-beta-hydroxysteroid dehydrogenase activity; biological_process unknown; metabolism; microsome; oxidoreductase activity; steroid metabolism
AA193553	-1.4141				
AA023029	-1.4229	PPP5C	5536	protein phosphatase 5, catalytic subunit	
H62618	-1.424				
R76163	-1.4362	ZYX	7791	zyxin	cell adhesion; cell adhesion molecule activity; cell-cell signaling; focal adhesion; integral to plasma membrane; plasma membrane; signal transduction
H01987	-1.4431	MLL3	58508	myeloid/lymphoid or mixed-lineage leukemia3	

N29429	-1.5061	CGI-57	27013	hypothetical protein CGI-57	
AA032288	-1.6506				
R72685	-1.6521	PLD3	23646	phospholipase D3	catalytic activity; metabolism; phospholipase D activity
N65982	-1.6535				
W68050	-1.6844	LGALS1	3956	lectin, galactoside-binding, soluble, 1 (galectin 1)	apoptosis; heterophilic cell adhesion; sugar binding
H49989	-1.7217	BOCT	51310	potent brain type organic ion transporter	integral to membrane; transport; transporter activity
H26465	-1.7737	GSN	2934	gelsolin (amyloidosis, Finnish type)	actin cytoskeleton; actin filament polymerization; actin filament severing activity; barbed-end actin capping/severing activity; calcium ion binding; cytosol; extracellular; structural constituent of cytoskeleton
R13936	-1.7787	DHPS	1725	deoxyhypusine synthase	deoxyhypusine synthase activity; hypusine biosynthesis from peptidyl-lysine; positive regulation of cell proliferation; protein biosynthesis; transferase activity
H24956	-1.844	RET	5979	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	oncogenesis; posterior midgut development; protein amino acid phosphorylation; protein tyrosine kinase activity; receptor activity; signal transduction
W69432	-1.8457	DDIT3	1649	DNA-damage inducible transcript 3	Transcription co-repressor activity; transcription factor activity; cell cyle arrest; response to DNA damage stimulus; cell growth and/or maintenance; regulation of transcription, DNA-dependent; nucleus

R00207	-2.214	SLC22A3	6581	solute carrier family 22 (extraneuronal monoamine transporter), member 3	integral to plasma membrane; ion transport; ion transporter activity; membrane fraction; organic cation transport; organic cation transporter activity
H86199	-2.2282				
H19719	-2.4026	GEMIN5	25929	gem (nuclear organelle) associated protein 5	
N52375	-4.3698				

Appendix B. Genes Differentially Regulated by 15m MPP+ and 15m MPP+/PTIO in Combination.

GENBANK	P VALUE	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
H01987	8.26E-10	MLL3	58508	myeloid/lymphoid or mixed-lineage leukemia3	
H09869	3.24E-07	GNAI2	2771	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	G-protein coupled receptor protein signaling pathway; GTP binding; heterotrimeric G-protein GTPase activity; negative regulation of adenylate cyclase activity; response to nutrients; signal transducer activity; signal transduction
W19744	8.06E-07				
R07186	2.21E-06				
AA037284	2.26E-06	APRT	353	adenine phosphoribosyltransferase	adenine phosphoribosyltransferase activity; adenine salvage pathway; nucleoside metabolism; transferase activity, transferring glycosyl groups
H59405	3.27E-06	FLJ10298	54682	hypothetical protein FLJ10298	
H51834	3.35E-06	TTC1	7265	tetratricopeptide repeat domain 1	chaperone activity; protein binding; protein folding
T86338	5.85E-06				
R76163	1.15E-05	ZYX	7791	zyxin	cell adhesion; cell adhesion molecule activity; cell-cell signaling; focal adhesion; integral to plasma membrane; plasma membrane; signal transduction
H68587	4.89E-05		340833	LOC340833	

W52537	6.17E-05	PSMA2	5683	proteasome (prosome, macropain) subunit, alpha type, 2	26S proteasome; cytosol; endopeptidase activity; proteasome core complex (sensu Eukarya); proteasome endopeptidase activity; ubiquitin-dependent protein catabolism
H26552	8.54E-05	MGC5395	79026	hypothetical protein MGC5395	intracellular signaling cascade
R20373	9.30E-05	TMP21	10972	transmembrane trafficking protein	ER to Golgi transport; Golgi apparatus; integral to plasma membrane; intracellular protein transport; membrane fraction; microsome; protein carrier activity; protein transporter activity
W68050	9.91E-05	LGALS1	3956	lectin, galactoside-binding, soluble, 1 (galectin 1)	apoptosis; heterophilic cell adhesion; sugar binding
H67193	0.000143814	EIF2S3	1968	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	GTPase activity; cytosolic small ribosomal subunit (sensu Eukarya); eukaryotic translation initiation factor 2 complex; translation elongation factor activity; translation initiation factor activity; translational elongation
R22402	0.000145207				
AA040852	0.000146241	KIAA1321	57532	KIAA1321 protein	
R51354	0.000171049	CNTNAP2	26047	contactin associated protein-like 2	
AA069448	0.000177002				
N95545	0.000178172	IL11	3589	interleukin 11	B-cell differentiation; adipocyte differentiation; cell proliferation; cell-cell signaling; cytokine activity; extracellular; interleukin-11 receptor binding; megakaryocyte differentiation; platelet activation; positive

					regulation of cell proliferation
R94499	0.000233405	GNB5	10681	guanine nucleotide binding protein (G protein), beta 5	
W01319	0.000249061	BHC80	51317	BRAF35/HDAC2 complex (80 kDa)	
R13346	0.0003047	ENT4	222962	equilibrative nucleoside transporter 4	
W47153	0.000339402	PTRF	284119	polymerase I and transcript release factor	
AA131933	0.000353436	ABP1	26	amiloride binding protein 1 (amine oxidase (copper-containing))	amine oxidase (copper-containing) activity; copper ion binding; drug binding; heparin binding; metabolism; oxidoreductase activity; peroxisome
R89790	0.000381932				
W85877	0.000608409				
AA101859	0.000631871	ENSA	2029	endosulfine alpha	ion channel inhibitor activity; receptor binding; response to nutrients; transport
N71628	0.000686384	SPIB	6689	Spi-B transcription factor (Spi-1/PU.1 related)	RNA polymerase II transcription factor activity; biological_process unknown; cytoplasm; molecular_function unknown; nucleus; regulation of transcription from Pol II promoter; transcription factor activity
R98517	0.000726742	HIST1H1C	3006	histone 1, H1c	DNA binding; chromosome; chromosome organization and biogenesis (sensu Eukarya); nucleosome; nucleosome assembly; nucleus
H63763	0.000872181				
H65775	0.000893253				

AA213450	0.000934614				
AA035066	0.001077269	MGC4268	83607	hypothetical protein MGC4268	
R47859	0.001191811	NPR1	4881	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	ATP binding; cGMP biosynthesis; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; peptide receptor activity, G-protein coupled; protein amino acid phosphorylation; protein kinase activity; receptor activity; receptor guanylate cyclase activity; regulation of blood pressure
AA004845	0.001331715	KIAA1529	57653	KIAA1529 protein	
AA002135	0.001365198	C2	717	complement component 2	chymotrypsin activity; classical-complement pathway C3/C5 convertase activity; complement activation, classical pathway; complement component C2 complex; hydrolase activity; proteolysis and peptidolysis; trypsin activity
H64569	0.001442673				
AA031859	0.001463558	TIMM13	26517	translocase of inner mitochondrial membrane 13 homolog (yeast)	hearing; mitochondrial inner membrane pre-sequence translocase complex; mitochondrial translocation; mitochondrion; protein targeting; protein translocase activity; zinc ion binding
W69432	0.001627691	MAPKAPK2	9261	mitogen-activated protein kinase-activated protein kinase 2	ATP binding; MAPKKK cascade; nucleus; protein amino acid phosphorylation; protein serine/threonine kinase activity; signal

					transducer activity; transferase activity
AA135646	0.001668986	hIAN6	155038	human immune associated nucleotide 6	
AA057286	0.002040228	TA-WDRP	134430	T-cell activation WD repeat protein	catalytic activity; metabolism
AA046245	0.00228188	OSF-2	10631	osteoblast specific factor 2 (fasciclin I-like)	cell adhesion; cell adhesion molecule activity; extracellular matrix; skeletal development
AA010141	0.002547572	SERPINH1	871	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	heat shock response
AA031564	0.002660207	LOC113444	113444	hypothetical protein BC011880	
N94432	0.002746068				
N90527	0.00279075	PIM1	5292	pim-1 oncogene	ATP binding; cAMP-dependent protein kinase activity; cell growth and/or maintenance; cytoplasm; development; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; transferase activity
AA046610	0.002804358				
N24815	0.002848453	UBA52	7311	ubiquitin A-52 residue ribosomal protein fusion product 1	nucleus; protein biosynthesis; protein modification; ribosome; structural constituent of ribosome
N22392	0.002974381	CLDN11	5010	claudin 11 (oligodendrocyte transmembrane protein)	integral to membrane; structural molecule activity; tight junction
AA054115	0.002987791				
R50905	0.003042081	TUBB	7280	tubulin, beta polypeptide	cytoskeleton; structural constituent of cytoskeleton
N39407	0.003085277	KIF21A	55605	kinesin family member 21A	
W60305	0.003247636				

R75598	0.003519312	NBL1	4681	neuroblastoma, suppression of tumorigenicity 1	negative regulation of cell cycle
H68885	0.003639707	TSSC3	7262	tumor suppressing subtransferable candidate 3	apoptosis; imprinting
AA129727	0.00378033	RAB5C	5878	RAB5C, member RAS oncogene family	GTP binding; RAB small monomeric GTPase activity; intracellular protein transport; protein transporter activity; small GTPase mediated signal transduction
AA151307	0.004494561	GNB2	2783	guanine nucleotide binding protein (G protein), beta polypeptide 2	G-protein coupled receptor protein signaling pathway; heterotrimeric G-protein GTPase activity; heterotrimeric G-protein complex; signal transducer activity; signal transduction
N48735	0.004677987				
N29429	0.004746315	CGI-57	27013	hypothetical protein CGI-57	
W04610	0.004828823	H3F3A	3020	H3 histone, family 3A	
W52156	0.005026981	OXTR	5021	oxytocin receptor	G-protein signaling, coupled to IP3 second messenger (phospholipase C activating); endosome; integral to plasma membrane; lactation; muscle contraction; oxytocin receptor activity; pregnancy; rhodopsin-like receptor activity; vasopressin receptor activity
AA204664	0.005257297	SMC1L2	27127	SMC1 structural maintenance of chromosomes 1-like 2 (yeast)	ATP binding; ATP-binding cassette (ABC) transporter activity; cell cycle; chromosome segregation; kinesin complex; meiosis; membrane; nucleus; transport
N34901	0.005737532	GALNT7	117248	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7	

AA098865	0.005823667	BCL2L10	10017	BCL2-like 10 (apoptosis facilitator)	anti-apoptosis; apoptosis inhibitor activity; caspase activation; integral to membrane; membrane fraction; mitochondrion; oogenesis; protein binding; spermatogenesis

Appendix C. Genes Differentially Regulated by 15m MPP+ and 15m MPP+/SN50 in Combination.

GENBANK	P VALUE	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
T87888	4.54E-10	KIAA1046	22867	KIAA1046 protein	
R94499	9.77E-10	GNB5	10681	guanine nucleotide binding protein (G protein), beta 5	
H68885	9.58E-09	TSSC3	7262	tumor suppressing subtransferable candidate 3	apoptosis; imprinting
R19119	1.01E-08				
AA010141	1.23E-08	SERPINH1	871	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	heat shock response
H18190	2.29E-08	JAK1	3716	Janus kinase 1 (a protein tyrosine kinase)	ATP binding; cytoskeleton; intracellular signaling cascade; protein amino acid phosphorylation; protein tyrosine kinase activity; transferase activity
H18298	4.04E-08				
H84257	7.13E-08				
H25578	7.76E-08				
R17538	2.15E-07	PABPC4	8761	poly(A) binding protein, cytoplasmic 4 (inducible form)	RNA binding; RNA catabolism; RNA processing; blood coagulation; cytoplasm; poly(A) binding; protein biosynthesis; response to pest/pathogen/parasite
H58631	2.48E-07				
R41363	2.58E-07				
H65775	5.19E-07				
R48610	6.16E-07	TTC7	57217	tetratricopeptide repeat domain 7	
H18495	7.34E-07				
H45355	2.47E-06				
W68050	2.54E-06	LGALS1	3956	lectin, galactoside-binding, soluble,	apoptosis; heterophilic cell adhesion; sugar binding

				1 (galectin 1)	
H68441	3.08E-06	FLJ14054	79614	hypothetical protein FLJ14054	
H29730	3.44E-06				
R20373	3.78E-06	TMP21	10972	transmembrane trafficking protein	ER to Golgi transport; Golgi apparatus; integral to plasma membrane; intracellular protein transport; membrane fraction; microsome; protein carrier activity; protein transporter activity
H09945	8.07E-06				
H19297	8.11E-06	EDIL3	10085	EGF-like repeats and discoidin I-like domains 3	calcium ion binding; cell adhesion; cell adhesion molecule activity; development; integrin binding
H23933	8.56E-06				
R47938	9.78E-06	FLJ32096	148646	hypothetical protein FLJ32096	
H27352	9.93E-06	HRAS	3265	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	GTPase activity; cell motility; cell shape and cell size control; cell surface receptor linked signal transduction; chemotaxis; cytoplasm; histogenesis and organogenesis; peripheral plasma membrane protein; plasma membrane; regulation of cell cycle; signal transduction
R85191	9.96E-06	FLJ31364	146956	homolog of yeast EME1 endonuclease	
R07186	1.18E-05				
H00498	1.20E-05	PPP2R3A	5523	protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha	calcium ion binding; protein phosphatase type 2A, intrinsic regulator activity
H84293	1.58E-05	SLC12A5	57468	solute carrier family 12, (potassium-chloride transporter) member 5	amino acid transport; amino acid-polyamine transporter activity; cell ion homeostasis; chloride transport; integral to membrane; ion transport; potassium ion transport; potassium:chloride symporter activity; sodium ion transport; symporter activity; transporter activity
N29429	1.77E-05	CGI-57	27013	hypothetical protein CGI-57	
R26844	1.90E-05				

H47146	2.57E-05	ERCC1	2067	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	DNA repair; embryogenesis and morphogenesis; endodeoxyribonuclease activity; nucleotide-excision repair; nucleus
H26760	4.12E-05	KIAA0375	9853	KIAA0375 gene product	
H27334	4.54E-05	DDR1	780	discoidin domain receptor family, member 1	ATP binding; cell adhesion; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; transferase activity; transmembrane receptor protein tyrosine kinase activity; transmembrane receptor protein tyrosine kinase signaling pathway
T98139	5.04E-05	HLA-B	3106	major histocompatibility complex, class I, B	MHC class I receptor activity; antigen presentation, endogenous antigen; antigen processing, endogenous antigen via MHC class I; immune response; integral to plasma membrane
R21970	5.86E-05	GTF2H2	2966	general transcription factor IIH, polypeptide 2, 44kDa	DNA repair; nucleus; regulation of transcription, DNA-dependent
R50087	6.67E-05	GREB1	9687	GREB1 protein	
H86672	7.16E-05				
H70974	7.39E-05				
H84008	8.89E-05				
R44307	0.000103006	PPP1R9B	84687	protein phosphatase 1, regulatory subunit 9B, spinophilin	intracellular signaling cascade; membrane; transport; transporter activity
H83405	0.000104317	FGD1	2245	faciogenital dysplasia (Aarskog-Scott syndrome)	development; guanyl-nucleotide exchange factor activity; histogenesis and organogenesis; signal transduction; zinc ion binding
H40607	0.00010928				

AA031859	0.000109417	TIMM13	26517	translocase of inner mitochondrial membrane 13 homolog (yeast)	hearing; mitochondrial inner membrane pre-sequence translocase complex; mitochondrial translocation; mitochondrion; protein targeting; protein translocase activity; zinc ion binding
H20790	0.000123136		348024	similar to TPIP alpha lipid phosphatase	
R72577	0.00017949	FLJ11753	79712	hypothetical protein FLJ11753	
H52741	0.000188793				
R89056	0.000191859	LAMP1	3916	lysosomal-associated membrane protein 1	integral to plasma membrane; lysosome; membrane fraction
R22402	0.000194653				
H02088	0.000194866	RBAF600	23352	retinoblastoma-associated factor 600	
H82992	0.000210935	PIGT	51604	phosphatidyl inositol glycan class T	
H49225	0.00035608				
H52939	0.000374234				
R54918	0.000382138	FLJ13912	64785	hypothetical protein FLJ13912	
R28090	0.000402226	KIAA1495	57631	KIAA1495 protein	
N45640	0.000411849	CH25H	9023	cholesterol 25-hydroxylase	catalytic activity; lipid metabolism; membrane fraction; steroid hydroxylase activity
H27034	0.000427779	IGKC	3514	immunoglobulin kappa constant	antigen binding; immune response
R23351	0.00045636				
R88435	0.000463204	DPP6	1804	dipeptidylpeptidase 6	catalytic activity; dipeptidyl-peptidase IV activity; dipeptidyl-peptidase activity; integral to membrane; proteolysis and peptidolysis
H45746	0.000474872				
R85044	0.00049781	SMPD1	6609	sphingomyelin phosphodiesterase 1, acid lysosomal (acid sphingomyelinase)	carbohydrate metabolism; hydrolase activity, acting on glycosyl bonds; lysosome; neurogenesis; signal transduction; sphingomyelin metabolism; sphingomyelin phosphodiesterase activity
H63763	0.000624428				

H47026	0.000674021	MGAT3	4248	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	Golgi apparatus; N-linked glycosylation; beta-1,4-mannosylglycoprotein beta-1,4-N-acetylglucosaminyltransferase activity; integral to membrane; transferase activity, transferring glycosyl groups
N80976	0.000700273	LOC51252	51252	hypothetical protein LOC51252	
R85150	0.000724255	EPHB6	2051	EphB6	ATP binding; ephrin receptor activity; integral to membrane; protein amino acid phosphorylation; protein tyrosine kinase activity; receptor activity; transmembrane receptor protein tyrosine kinase signaling pathway
H26552	0.001011054	MGC5395	79026	hypothetical protein MGC5395	intracellular signaling cascade
H43455	0.001071695	PP2447	80305	hypothetical protein PP2447	
H93450	0.001256746	ZNF347	84671	zinc finger protein 347	DNA binding; nucleus; regulation of transcription, DNA-dependent
H71213	0.001430042	F2	2147	coagulation factor II (thrombin)	STAT protein nuclear translocation; acute-phase response; apoptosis; blood coagulation; calcium ion binding; caspase activation; chymotrypsin activity; development; extracellular space; hydrolase activity; proteolysis and peptidolysis; regulation of cell cycle; response to wounding; soluble fraction; thrombin activity; trypsin activity; tyrosine phosphorylation of STAT protein
N25523	0.001869222	HSPE1	3336	heat shock 10kDa protein 1 (chaperonin 10)	co-chaperonin activity; heat shock protein activity; mitochondrion; protein folding
R49189	0.001912823	SLC30A6	55676	solute carrier family 30 (zinc transporter), member 6	
H59454	0.002111161				
R48615	0.002214369	C14orf21	161424	chromosome 14 open reading frame 21	RNA binding

H83025	0.002258751				
H46133	0.002673406	BAI2	576	brain-specific angiogenesis inhibitor 2	G-protein coupled receptor activity; integral to membrane; neuropeptide signaling pathway
N39391	0.002748964	MGC14799	84296	hypothetical protein MGC14799	
R47859	0.002825359	NPR1	4881	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	ATP binding; cGMP biosynthesis; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; peptide receptor activity, G-protein coupled; protein amino acid phosphorylation; protein kinase activity; receptor activity; receptor guanylate cyclase activity; regulation of blood pressure
H59405	0.002940249	FLJ10298	54682	hypothetical protein FLJ10298	
AA031950	0.003182394				
R39421	0.003373338	PIGM	93183	phosphatidylinositol glycan, class M	transferase activity
T84788	0.003432787				
R82834	0.00345738				
H20520	0.003478042				
H69440	0.003723647	ANKRD13	88455	ankyrin repeat domain 13	
H69011	0.003868629	SKIL	6498	SKI-like	cell differentiation; cell growth and/or maintenance; molecular_function unknown; nucleus
H45972	0.003905578				
R90824	0.003908283	TMEM10	93377	transmembrane protein 10	integral to membrane
H51160	0.004249526	PPP2R1A	5518	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	protein phosphatase type 2A activity
AA037284	0.004277598	APRT	353	adenine phosphoribosyltransferase	adenine phosphoribosyltransferase activity; adenine salvage pathway; nucleoside metabolism; transferase activity, transferring glycosyl groups

H04530	0.004311455	ECHS1	1892	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	energy pathways; fatty acid beta-oxidation; fatty acid metabolism; long-chain enoyl-CoA hydratase activity; lyase activity; mitochondrion; short-chain enoyl-CoA hydratase activity
H62770	0.004355849				
T86338	0.004449624				
R88711	0.004475335				

Appendix D. Genes that change +/- 2 fold in response to 90m MPP+.

GENBANK	+/- LOG_2	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
AA045373	1.441517459	TCEAL1	9338	transcription elongation factor A (SII)-like 1	RNA polymerase II transcription factor activity; negative regulation of transcription from Pol II promoter; nucleus; regulation of transcription, DNA-dependent; transcription factor activity; translation elongation factor activity
H69656	1.403296938	NARF	26502	nuclear prelamin A recognition factor	lamin binding; nuclear lamina
H68373	1.381707958	TFCP2	7024	transcription factor CP2	DNA binding; regulation of transcription from Pol II promoter; transcription factor activity
H60376	1.326456548				
H66920	1.323418429				
H62770	1.313201378				
H61030	1.291270823				
H61972	1.278225142	PIN4	5303	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	FK506-sensitive peptidyl-prolyl cis-trans isomerase; cyclophilin; cyclophilin-type peptidy-prolyl cis-trans isomerase activity; isomerase activity; mitochondrial matrix; protein folding
N30939	1.269259316				
H62766	1.26421627				
H63794	1.263829666	NFATC1	4772	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	FK506 binding; cytoplasm; nucleus; regulation of transcription, DNA-dependent; transcription factor activity; transcription from Pol II promoter
H48676	1.263058048				
H60458	1.252815748	ACOX2	8309	acyl-Coenzyme A oxidase 2, branched chain	acyl-CoA oxidase activity; bile acid metabolism; electron transport; fatty acid beta-oxidation; fatty acid metabolism; oxidoreductase activity; peroxisome
W86443	1.24926473				
H45355	1.245053836				
H09945	1.241279937				

H62473	1.23886115	TGFBR3	7049	transforming growth factor, beta receptor III (betaglycan, 300kDa)	TGFbeta receptor signaling pathway; development; glycosaminoglycan binding; integral to membrane; receptor activity; signal transduction
H84096	1.233985965				
H70485	1.233118474	MBNL3	55796	muscleblind-like 3 (Drosophila)	development; nucleic acid binding; nucleus
R96672	1.229567068	CYP2D6	1565	cytochrome P450, family 2, subfamily D, polypeptide 6	cytochrome P450 activity
H60340	1.218704746				
H61974	1.213195891				
H59062	1.20663091	KIAA0602	23241	KIAA0602 protein	
W47000	1.205992418	HAK	115701	heart alpha-kinase	
AA045817	1.205511408	MAGEA8	4107	melanoma antigen, family A, 8	biological_process unknown; cellular_component unknown; molecular_function unknown
AA136884	1.204444126	FLJ21924	79832	hypothetical protein FLJ21924	
AA099373	1.204236905	GYS1	2997	glycogen synthase 1 (muscle)	glycogen metabolism
H51160	1.203703177	PPP2R1A	5518	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	protein phosphatase type 2A activity
H82521	1.195709551	ATP6V0B	533	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit c''	ATP biosynthesis; hydrogen ion transporter activity; hydrogen-transporting two-sector ATPase activity; hydrolase activity; integral to membrane; proton transport; transporter activity
AA029583	1.195391457	TFF3	7033	trefoil factor 3 (intestinal)	defense response; digestion; extracellular
W86198	1.189144525	KIAA0905	22872	yeast Sec31p homolog	
H83003	1.186074391	IGSF1	3547	immunoglobulin superfamily, member 1	cell adhesion; integral to plasma membrane
R85183	1.181843544	C20orf98	80023	chromosome 20 open reading frame 98	integral to membrane
AA142989	1.173727565	BMPER	168667	likely ortholog of mouse BMP-binding endothelial regulator precursor	calcium ion binding; extracellular

				protein	
H59810	1.165111447	CLU	1191	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	apoptosis; cell death; complement activation, classical pathway; fertilization (sensu Animalia); lipid metabolism
H67584	1.163534475				
R85191	1.15964466	FLJ31364	146956	homolog of yeast EME1 endonuclease	
W58177	1.158467769	HIST2H2AA	8337	histone 2, H2aa	DNA binding; chromosome; chromosome organization and biogenesis (sensu Eukarya); nucleosome; nucleosome assembly; nucleus
H62266	1.154316023				
AA044141	1.151517188	C20orf98	80023	chromosome 20 open reading frame 98	integral to membrane
H68441	1.149340598	FLJ14054	79614	hypothetical protein FLJ14054	
N45013	1.142586597				
H65775	1.140757184				
H18298	1.13931833				
H68440	1.129174679	PIP5K1B	8395	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	
AA115377	1.129084061	TMPO	7112	thymopoietin	lamin binding; lamin/chromatin binding; nuclear membrane; nucleus
R21970	1.128566868	GTF2H2	2966	general transcription factor IIH, polypeptide 2, 44kDa	DNA repair; nucleus; regulation of transcription, DNA-dependent
H29730	1.127973609				
H68949	1.121067809				
AA059131	1.120131935	BTBD1	53339	BTB (POZ) domain containing 1	biological_process unknown; cellular_component unknown; protein binding
H60520	1.117545032				
H43455	1.114606867	PP2447	80305	hypothetical protein PP2447	
AA147503	1.113307029	RRS1	23212	RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)	nucleus; ribosome biogenesis

T51698	1.111278341				
H18495	1.108995666				
R89056	1.105715702	LAMP1	3916	lysosomal-associated membrane protein 1	integral to plasma membrane; lysosome; membrane fraction
H47026	1.103738337	MGAT3	4248	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	Golgi apparatus; N-linked glycosylation; beta-1,4-mannosylglycoprotein beta-1,4-N-acetylglucosaminyltransferase activity; integral to membrane; transferase activity, transferring glycosyl groups
AA037661	1.101101359	C21orf70	85395	chromosome 21 open reading frame 70	
H68441	1.09871499	FLJ14054	79614	hypothetical protein FLJ14054	
R21373	1.098435204	HMGN1	3150	high-mobility group nucleosome binding domain 1	DNA binding; RNA polymerase II transcription factor activity; chromatin; positive transcription elongation factor activity
AA151360	1.091171256	ARHGAP12	94134	Rho GTPase activating protein 12	
AA054541	1.090113332				
AA203677	1.087790185				
R85333	1.087525971				
AA047517	1.086114591	VRK3	51231	vaccinia related kinase 3	ATP binding; protein amino acid phosphorylation; protein kinase activity; transferase activity
R12665	1.084961201		197135	similar to RIKEN cDNA 4930424G05	
N80432	1.084518406				
W39129	1.081123099	NUCB1	4924	nucleobindin 1	DNA binding; Golgi apparatus; calcium ion binding; extracellular space
N48003	1.079307263	MGC9912	112487	similar to RIKEN cDNA 4930578F06 gene	D-amino acid catabolism; cytoplasm; hydrolase activity, acting on ester bonds
H65231	1.077877352				
H39058	1.07691367				
H67094	1.075796998				
AA149233	1.075759317	PTGES	9536	prostaglandin E synthase	antimicrobial humoral response (sensu Invertebrata); membrane fraction; prostaglandin

					metabolism; signal transduction
AA098907	1.074777518	ALAS1	211	aminolevulinate, delta-, synthase 1	5-aminolevulinate synthase activity; acyltransferase activity; biosynthesis; heme biosynthesis; mitochondrion; transaminase activity; transferase activity
H93445	1.073357733				
H38321	1.072843358	FLJ14360	84861	hypothetical protein FLJ14360	protein binding
H48589	1.068620209	HSPA4	3308	heat shock 70kDa protein 4	molecular_function
H66835	1.068287191				
H68976	1.062900057	BXDC1	84154	brix domain containing 1	nucleus
AA058835	1.060918908				
N52765	1.059040178	CACNA1I	8911	calcium channel, voltage-dependent, alpha 1I subunit	calcium ion binding; calcium ion transport; cation transport; integral to membrane; low voltage-gated calcium channel activity; voltage-gated calcium channel complex
AA134752	1.055574306	SLC30A5	64924	solute carrier family 30 (zinc transporter), member 5	
H70468	1.054468231				
N94749	1.054030574				
H68952	1.051992045	ITGA1	3672	integrin, alpha 1	cell adhesion receptor activity; cell-matrix adhesion; collagen binding; integral to membrane; integrin complex; integrin-mediated signaling pathway; magnesium ion binding; receptor activity
H68718	1.051870928	ROCK1	6093	Rho-associated, coiled-coil containing protein kinase 1	ATP binding; Rho protein signal transduction; actin cytoskeleton organization and biogenesis; intracellular; intracellular signaling cascade; protein amino acid phosphorylation; protein serine/threonine kinase activity; protein tyrosine kinase activity; transferase activity
H62909	1.049977564				
R47938	1.049354437	FLJ32096	148646	hypothetical protein FLJ32096	

H24891	1.047426291				
N40017	1.046865373	MRPL24	79590	mitochondrial ribosomal protein L24	intracellular; protein biosynthesis; ribosome; structural constituent of ribosome
W55993	1.046467205	FBN2	2201	fibrillin 2 (congenital contractural arachnodactyly)	calcium ion binding; embryogenesis and morphogenesis; extracellular matrix; extracellular matrix structural constituent; histogenesis and organogenesis
H09701	1.045444795				
H48578	1.045334733				
H69787	1.044928552				
N27190	1.044272505	UCHL3	7347	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)	cytoplasm; hydrolase activity; ubiquitin C-terminal hydrolase activity; ubiquitin-dependent protein catabolism
H61036	1.043982636				
AA099441	1.037648009	NUCB1	4924	nucleobindin 1	DNA binding; Golgi apparatus; calcium ion binding; extracellular space
R87198	1.037149735	TUBB5	10382	tubulin, beta, 5	cytoskeleton; structural constituent of cytoskeleton
N78467	1.035593133	PWP1	11137	nuclear phosphoprotein similar to S. cerevisiae PWP1	nucleus; transcription
R90824	1.034623848	TMEM10	93377	transmembrane protein 10	integral to membrane
AA099636	1.03442035	KIAA1039	23108	KIAA1039 protein	
R31364	1.034277696	LOC283337	283377	hypothetical protein LOC283377	
H27034	1.033434891	IGKC	3514	immunoglobulin kappa constant	antigen binding; immune response
W86640	1.032117754	RIPK1	8737	receptor (TNFRSF)-interacting serine-threonine kinase 1	ATP binding; apoptosis; cAMP-dependent protein kinase activity; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; protein tyrosine kinase activity; signal transduction; transferase activity
R48311	1.030542722	FLJ38608	132228	hypothetical protein FLJ38608	

H62477	1.029641741	CD36	948	CD36 antigen (collagen type I receptor, thrombospondin receptor)	blood coagulation; cell adhesion; cell adhesion molecule activity; fatty acid metabolism; integral to plasma membrane; membrane fraction; receptor activity; transport
H94763	1.028965825	SH3GLB1	51100	SH3-domain GRB2-like endophilin B1	
H52253	1.027982817	IGHG3	3502	immunoglobulin heavy constant gamma 3 (G3m marker)	antigen binding; immune response; membrane fraction
W70111	1.025583613	ADH5	128	alcohol dehydrogenase 5 (class III), chi polypeptide	alcohol dehydrogenase activity, iron-dependent; alcohol dehydrogenase activity, metal ion-independent; alcohol dehydrogenase activity, zinc-dependent; alcohol metabolism; electron transporter activity; ethanol oxidation; fatty acid binding; formaldehyde dehydrogenase (glutathione) activity; oxidoreductase activity; zinc ion binding
H27352	1.024405897	HRAS	3265	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	GTPase activity; cell motility; cell shape and cell size control; cell surface receptor linked signal transduction; chemotaxis; cytoplasm; histogenesis and organogenesis; peripheral plasma membrane protein; plasma membrane; regulation of cell cycle; signal transduction
AA033714	1.022700441	FLJ14260	80095	hypothetical protein FLJ14260	DNA binding; metallopeptidase activity; nucleus; proteolysis and peptidolysis; regulation of transcription, DNA-dependent; zinc ion binding
AA029775	1.021800833	FUS1	11334	lung cancer candidate	cell proliferation; cell-cell signaling; negative regulation of cell cycle
AA152304	1.021453662	ARF3	377	ADP-ribosylation factor 3	Golgi apparatus; intracellular protein transport; nonselective vesicle assembly; protein transporter activity; small

					GTPase mediated signal transduction; small monomeric GTPase activity
R07186	1.019988898				
AA148416	1.019647544	SERAC1	84947	serine active site containing 1	catalytic activity
R27269	1.019572266				
H56304	1.019388908	ENTPD1	953	ectonucleoside triphosphate diphosphohydrolase 1	antimicrobial humoral response (sensu Invertebrata); apyrase activity; blood coagulation; cell adhesion; cell-cell signaling; hydrolase activity; integral to plasma membrane; magnesium ion binding; membrane fraction
R90757	1.018109648	RPH3A	22895	likely ortholog of mouse rabphilin 3A	intracellular protein transport; membrane; protein transporter activity; synaptic junction; synaptic vesicle; zinc ion binding
R41363	1.017230382				
T51972	1.017109657	SEMA4C	54910	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	development; membrane; receptor activity
AA058632	1.015551086	KIF1B	23095	kinesin family member 1B	
AA035437	1.01352019				
H86672	1.012957874				
H60542	1.011644443				
N89894	1.010068232				
AA057466	1.008653468				
H47518	1.008141366				
W47003	1.008005073	HIF1A	3091	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	
R01799	1.00741527				
N49763	1.007208746	WTAP	9589	Wilms tumor 1 associated protein	
AA009926	1.006656423				
H68528	1.003205293	FLJ32499	124637	hypothetical protein FLJ32499	
H62045	1.00061111	LOC117584	117584	fring	
H10658	1.000551286				

AA059148	1.000473754	KIAA1199	57214	KIAA1199 protein	
AA057126	1.000325845	KIAA1416	55636	KIAA1416 protein	
N91341	-1.00337743				
T62916	-1.00877983	RPS29	6235	ribosomal protein S29	RNA binding; cytosolic small ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; ribosome; structural constituent of ribosome; zinc ion binding
N32235	-1.01486015	IGFBP4	3487	insulin-like growth factor binding protein 4	DNA metabolism; cell proliferation; extracellular; insulin-like growth factor binding; regulation of cell growth; signal transduction; skeletal development
N39407	-1.017239042	KIF21A	55605	kinesin family member 21A	
N29429	-1.023442466	CGI-57	27013	hypothetical protein CGI-57	
N91501	-1.052425513				
W32438	-1.107245642	CRABP2	1382	cellular retinoic acid binding protein 2	epidermal differentiation; lipid binding; regulation of transcription, DNA-dependent; retinoid binding; signal transduction; transport; transporter activity

Appendix E. Genes That Change +/- 2 fold at both 15m and 90m of MPP+ treatment.

GENBANK	SYMBOL	LOCUSLINK	GENE NAME
AA059148	KIAA1199	57214	KIAA1199 protein
AA057126	KIAA1416	55636	KIAA1416 protein
H59810	CLU	1191	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)
R96672	CYP2D6	1565	cytochrome P450, family 2, subfamily D, polypeptide 6
R21970	GTF2H2	2966	general transcription factor IIH, polypeptide 2, 44kDa
W58177	HIST2H2AA	8337	histone 2, H2aa
R85191	FLJ31364	146956	homolog of yeast EME1 endonuclease
N29429	CGI-57	27013	hypothetical protein CGI-57
H68441	FLJ14054	79614	hypothetical protein FLJ14054
H38321	FLJ14360	84861	hypothetical protein FLJ14360
R47938	FLJ32096	148646	hypothetical protein FLJ32096
H43455	PP2447	80305	hypothetical protein PP2447
H52253	IGHG3	3502	immunoglobulin heavy constant gamma 3 (G3m marker)
H27034	IGKC	3514	immunoglobulin kappa constant
H83003	IGSF1	3547	immunoglobulin superfamily, member 1
AA058632	KIF1B	23095	kinesin family member 1B
N39407	KIF21A	55605	kinesin family member 21A
R90757	RPH3A	22895	likely ortholog of mouse rabphilin 3A
R89056	LAMP1	3916	lysosomal-associated membrane protein 1
H47026	MGAT3	4248	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase
N40017	MRPL24	79590	mitochondrial ribosomal protein L24
N78467	PWP1	11137	nuclear phosphoprotein similar to S. cerevisiae PWP1
H69656	NARF	26502	nuclear prelamin A recognition factor
H51160	PPP2R1A	5518	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform
AA045373	TCEAL1	9338	transcription elongation factor A (SII)-like 1
R90824	TMEM10	93377	transmembrane protein 10
H27352	HRAS	3265	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
H09945			
H18298			
H18495			

H29730			
H45355			
H62766			
H62770			
H65775			
H86672			
N45013			
R07186			
R41363			
R85333			

Appendix F. Genes significantly different between 15m MPP+ & 15m MPP+ with PTIO.

GENBANK	PVALUE	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
H01987	8.26E-10	MLL3	58508	myeloid/lymphoid or mixed-lineage leukemia3	
H84325	1.88E-09	PBX3	5090	pre-B-cell leukemia transcription factor 3	DNA binding; anterior compartment specification; oncogenesis; posterior compartment specification
R37089	2.11E-09	PABPC1	26986	poly(A) binding protein, cytoplasmic 1	RNA binding; cytoplasm; mRNA polyadenylation; poly(A) binding
R09196	2.50E-09				
H71562	3.80E-09	LOC221303	221303	hypothetical protein LOC221303	
R26954	5.64E-08	CTSD	1509	cathepsin D (lysosomal aspartyl protease)	cathepsin D activity; hydrolase activity; lysosome; pepsin A activity; proteolysis and peptidolysis
R07461	7.99E-08				
R01950	8.77E-08				
H80104	1.20E-07				
AA047309	1.30E-07	LAMC2	3918	laminin, gamma 2	basement membrane; cell adhesion; cell adhesion molecule activity; epidermal differentiation; heparin binding; kinesin complex; laminin-5; structural molecule activity
R66080	2.65E-07	KIAA1530	57654	KIAA1530 protein	
H09869	3.24E-07	GNAI2	2771	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	G-protein coupled receptor protein signaling pathway; GTP binding; heterotrimeric G-protein GTPase activity; negative regulation of adenylate cyclase activity; response to nutrients; signal transducer activity; signal transduction
W32999	3.75E-07	RPS26	6231	ribosomal protein S26	RNA binding; cytosolic small ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; ribosome; structural constituent of ribosome

R99604	4.53E-07				
W19744	8.06E-07				
T96291	8.09E-07				
AA136363	9.79E-07				
N47077	1.27E-06	FLJ38973	205327	hypothetical protein FLJ38973	
R55491	1.30E-06				
N92911	1.62E-06	DJ473B4	56180	hypothetical protein dJ473B4	structural molecule activity
R07186	2.21E-06				
AA037284	2.26E-06	APRT	353	adenine phosphoribosyltransferase	adenine phosphoribosyltransferase activity; adenine salvage pathway; nucleoside metabolism; transferase activity, transferring glycosyl groups
H83488	2.48E-06				
R72441	3.24E-06	KIAA1223	57182	KIAA1223 protein	
H59405	3.27E-06	FLJ10298	54682	hypothetical protein FLJ10298	
AA031913	3.34E-06	LOC123803	123803	N-terminal Asn amidase	
H51834	3.35E-06	TTC1	7265	tetratricopeptide repeat domain 1	chaperone activity; protein binding; protein folding
N64198	3.62E-06				
R91005	5.68E-06				
T86338	5.85E-06				
R87345	5.98E-06	MGC2656	79414	hypothetical protein MGC2656	
R68921	6.03E-06	XPO1	7514	exportin 1 (CRM1 homolog, yeast)	cytoplasm; nuclear pore; nucleoplasm; protein transporter activity; protein-nucleus import, docking
AA058661	6.29E-06				
R81337	6.57E-06	LOC51159	51159	colon carcinoma related protein	
R39516	7.79E-06	DKFZP761N09121	57183	hypothetical protein DKFZp761N09121	
R08412	8.01E-06				
H83464	8.49E-06				

T85114	9.33E-06	DEF6	50619	differentially expressed in FDCP 6 homolog (mouse)	
R99892	9.58E-06				
H06382	1.01E-05				
R76163	1.15E-05	ZYX	7791	zyxin	cell adhesion; cell adhesion molecule activity; cell-cell signaling; focal adhesion; integral to plasma membrane; plasma membrane; signal transduction
N33577	1.23E-05		348396	similar to hypothetical protein FLJ20489	
W53003	1.51E-05	RNASEL	6041	ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent)	ATP binding; RNA binding; cellular_component unknown; endoribonuclease activity; hydrolase activity; protein amino acid phosphorylation; protein kinase activity
R10993	1.53E-05				
H83987	1.57E-05				
AA121937	1.72E-05				
R18850	1.74E-05	TTYH1	57348	tweety homolog 1 (Drosophila)	integral to membrane; iron ion transport; iron ion transporter activity
N90246	1.75E-05	EPHA1	2041	EphA1	ATP binding; ephrin receptor activity; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; signal transduction; transferase activity; transmembrane receptor protein tyrosine kinase signaling pathway
H47346	1.86E-05	KMO	8564	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	aromatic compound metabolism; electron transport; electron transporter activity; kynurenine 3-monooxygenase activity
H87118	1.98E-05	FUT4	2526	fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)	Golgi apparatus; carbohydrate metabolism; fucosyltransferase activity; integral to membrane; membrane fraction; protein amino acid glycosylation; transferase activity, transferring glycosyl groups
T51698	1.99E-05				
R26598	2.48E-05				

T72012	2.87E-05	SFTPA2	6436	surfactant, pulmonary-associated protein A2	extracellular space; heterophilic cell adhesion; lipid transporter activity; respiratory gaseous exchange; sugar binding; surfactant activity
H50015	3.10E-05				
AA047259	3.11E-05	EBAF	7044	endometrial bleeding associated factor (left-right determination, factor A; transforming growth factor beta superfamily)	TGFbeta receptor signaling pathway; cell growth; cell-cell signaling; development; oocyte axis determination; transforming growth factor-beta receptor binding
R15444	3.13E-05	LOC348235	348235	hypothetical protein LOC348235	
H49830	3.18E-05				
AA027831	3.53E-05				
R40412	3.55E-05	CTNND2	1501	catenin (cadherin-associated protein), delta 2 (neural plakophilin-related arm-repeat protein)	cell adhesion; cell adhesion molecule activity; cytoskeleton; development; kinesin complex; neuronal cell adhesion; structural molecule activity
AA045130	3.62E-05	FLJ10761	55224	hypothetical protein FLJ10761	biological_process unknown; cellular_component unknown; choline kinase activity; transferase activity
H03447	3.67E-05				
T78085	3.77E-05	LOC200008	200008	hypothetical protein LOC200008	electron transport; oxidoreductase activity
H21648	3.98E-05				
AA203189	4.03E-05	TSP50	29122	testes-specific protease 50	chymotrypsin activity; peptidase activity; proteolysis and peptidolysis; trypsin activity
H45241	4.15E-05	RPL41	6171	ribosomal protein L41	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); protein biosynthesis; structural constituent of ribosome
N34437	4.19E-05	CAS1	64921	O-	

				acetyltransferase	
T87319	4.25E-05	C6orf56	9749	chromosome 6 open reading frame 56	
R14112	4.27E-05	CYP1A1	1543	cytochrome P450, family 1, subfamily A, polypeptide 1	cytochrome P450 activity; electron transport; endoplasmic reticulum; membrane; microsome; monooxygenase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen
R12622	4.29E-05	FTSJ2	29960	FtsJ homolog 2 (E. coli)	methyltransferase activity; nucleus; rRNA processing; transferase activity
AA099441	4.65E-05	NUCB1	4924	nucleobindin 1	DNA binding; Golgi apparatus; calcium ion binding; extracellular space
R52315	4.65E-05	OCA2	4948	oculocutaneous albinism II (pink-eye dilution homolog, mouse)	L-tyrosine transporter activity; cytoplasm; eye pigment biosynthesis; eye pigment precursor transporter activity; integral to membrane; transporter activity
R26526	4.67E-05	BNC	646	basonuclin	epidermal differentiation; nucleus; positive regulation of cell proliferation; regulation of transcription, DNA-dependent; transcription factor activity
AA004424	4.74E-05				
R07137	4.81E-05	HIC2	23119	hypermethylated in cancer 2	DNA binding; negative regulation of transcription, DNA-dependent; nucleus; protein C-terminus binding
H68587	4.89E-05		340833	LOC340833	
R00907	4.91E-05	PLEKHG1	57480	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	
T80564	5.11E-05	NMNAT2	23057	nicotinamide nucleotide adenylyltransferase 2	
AA042800	5.17E-05				
H48570	5.37E-05				
H39927	5.71E-05				
R18927	5.76E-05				
T86133	5.95E-05				

H46116	5.96E-05	GNG13	51764	guanine nucleotide binding protein (G protein), gamma 13	G-protein coupled receptor protein signaling pathway; heterotrimeric G-protein GTPase activity; heterotrimeric G-protein complex; signal transducer activity; signal transduction
H12333	6.00E-05	TFPI2	7980	tissue factor pathway inhibitor 2	blood coagulation; extracellular matrix; extracellular matrix structural constituent; serine protease inhibitor activity
R24299	6.03E-05	Rab11-FIP3	9727	KIAA0665 gene product	Rab interactor activity; calcium ion binding
H10851	6.08E-05				
W52537	6.17E-05	PSMA2	5683	proteasome (prosome, macropain) subunit, alpha type, 2	26S proteasome; cytosol; endopeptidase activity; proteasome core complex (sensu Eukarya); proteasome endopeptidase activity; ubiquitin-dependent protein catabolism
R24727	6.17E-05	PTDSR	23210	phosphatidylserine receptor	
H24375	6.26E-05				
R56097	6.47E-05	P29	25949	GCIP-interacting protein p29	biological_process unknown; nucleus; protein binding
W32180	6.49E-05				
R62213	7.78E-05				
T99071	7.94E-05				
R37498	8.01E-05	RALB	5899	v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)	GTP binding; RAS small monomeric GTPase activity; signal transduction; small GTPase mediated signal transduction
H98856	8.45E-05	TCF12	6938	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)	DNA binding; RNA polymerase II transcription factor activity; development; immune response; muscle development; nucleus; regulation of transcription from Pol II promoter
H26552	8.54E-05	MGC5395	79026	hypothetical protein MGC5395	intracellular signaling cascade

W31358	8.63E-05				
R49740	8.75E-05	FBXO21	23014	F-box only protein 21	
H81214	8.99E-05				
H68203	9.18E-05				
R20373	9.30E-05	TMP21	10972	transmembrane trafficking protein	ER to Golgi transport; Golgi apparatus; integral to plasma membrane; intracellular protein transport; membrane fraction; microsome; protein carrier activity; protein transporter activity
AA033795	9.32E-05				
W47002	9.56E-05	NME2	4831	non-metastatic cells 2, protein (NM23B) expressed in	ATP binding; CTP biosynthesis; GTP biosynthesis; UTP biosynthesis; kinase activity; negative regulation of cell cycle; negative regulation of cell proliferation; nucleoside triphosphate biosynthesis; nucleoside-diphosphate kinase activity; nucleus; regulation of transcription, DNA-dependent; transcription factor activity; transferase activity
W91885	9.85E-05				
W68050	9.91E-05	LGALS1	3956	lectin, galactoside-binding, soluble, 1 (galectin 1)	apoptosis; heterophilic cell adhesion; sugar binding
H06803	0.000102328				
H73928	0.000104464	SEC61B	10952	protein translocation complex beta	endoplasmic reticulum; integral to membrane; nonselective vesicle transport; protein targeting; protein translocase activity
H10123	0.000106775	SHREW1	55966	transmembrane protein SHREW1	metabolism; oxidoreductase activity
R13021	0.00010834	FLJ10751	55222	hypothetical protein FLJ10751	
H86049	0.000112712				
W38655	0.000113189	GJA1	2697	gap junction protein, alpha 1, 43kDa (connexin 43)	cell-cell signaling; connexon channel activity; connexon complex; hearing; heart development; integral to plasma membrane; ion transporter activity; muscle contraction; transport
R41480	0.000116	EFCBP1	64168	EF hand	calcium ion binding

	081			calcium binding protein 1	
T75124	0.000124039	CGI-67	51104	CGI-67 protein	
R62986	0.000129157				
AA039600	0.000129217	FGFR4	2264	fibroblast growth factor receptor 4	ATP binding; FGF receptor signaling pathway; fibroblast growth factor receptor activity; integral to plasma membrane; protein amino acid phosphorylation; protein-tyrosine kinase activity; receptor activity; transferase activity
H02012	0.000129857				
R55379	0.000130606	TNFSF12	8742	tumor necrosis factor (ligand) superfamily, member 12	angiogenesis; immune response; induction of apoptosis; integral to plasma membrane; signal transduction; tumor necrosis factor receptor binding
R68004	0.000134931	PCBP2	5094	poly(rC) binding protein 2	DNA binding; RNA binding; cytoplasm; mRNA metabolism; nucleus; ribonucleoprotein complex
R36127	0.000137083	FLNB	2317	filamin B, beta (actin binding protein 278)	actin binding; actin cytoskeleton; actin cytoskeleton organization and biogenesis; cytoskeletal anchoring; integral to plasma membrane; membrane associated actin binding; signal transduction
H67193	0.000143814	EIF2S3	1968	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	GTPase activity; cytosolic small ribosomal subunit (sensu Eukarya); eukaryotic translation initiation factor 2 complex; translation elongation factor activity; translation initiation factor activity; translational elongation
R22402	0.000145207				
AA040852	0.000146241	KIAA1321	57532	KIAA1321 protein	
R38712	0.000152754				
N30228	0.000155195	KIAA1432	57589	KIAA1432 protein	
R83199	0.000155413				

H68718	0.000158676	ROCK1	6093	Rho-associated, coiled-coil containing protein kinase 1	ATP binding; Rho protein signal transduction; actin cytoskeleton organization and biogenesis; intracellular; intracellular signaling cascade; protein amino acid phosphorylation; protein serine/threonine kinase activity; protein-tyrosine kinase activity; transferase activity
H02328	0.000163034	SLC2A1	6513	solute carrier family 2 (facilitated glucose transporter), member 1	carbohydrate transport; glucose transport; glucose transporter activity; integral to membrane; membrane fraction; sugar porter activity; transporter activity
R43191	0.000163622				
R85093	0.000165446				
H20379	0.000166734	WDR22	8816	WD repeat domain 22	oncogenesis
T65261	0.000168202				
H05660	0.000168597	C18orf1	753	chromosome 18 open reading frame 1	biological_process unknown; integral to membrane; molecular_function unknown
R51354	0.000171049	CNTNAP2	26047	contactin associated protein-like 2	
AA147654	0.000171844				
N26917	0.000171923				
R45094	0.000173118	ME3	10873	malic enzyme 3, NADP(+)-dependent, mitochondrial	electron transporter activity; malate dehydrogenase (oxaloacetate-decarboxylating) (NADP) activity; malate metabolism; mitochondrion; oxidoreductase activity; pyruvate metabolism
H43658	0.000174592	TRIM47	91107	tripartite motif-containing 47	intracellular; zinc ion binding
R81715	0.000175054	ATF4	468	activating transcription factor 4 (tax-	DNA binding; RNA polymerase II transcription factor activity; nucleus; regulation of transcription, DNA-dependent

				responsive enhancer element B67)	
AA069448	0.000177002				
N95545	0.000178172	IL11	3589	interleukin 11	B-cell differentiation; adipocyte differentiation; cell proliferation; cell-cell signaling; cytokine activity; extracellular; interleukin-11 receptor binding; megakaryocyte differentiation; platelet activation; positive regulation of cell proliferation
R51914	0.00018031	CGI-87	51112	CGI-87 protein	
H10439	0.000188608		338598	similar to hypothetical protein MGC5560	
R64301	0.000189177				
H38881	0.000190477	WHSC1	7468	Wolf-Hirschhorn syndrome candidate 1	embryogenesis and morphogenesis; oncogenesis
AA037091	0.000195333	TCEB1	6921	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)	nucleus; protein binding; regulation of transcription from Pol II promoter; transcriptional elongation regulator activity
N39397	0.000198774	BACH	11332	brain acyl-CoA hydrolase	acyl-CoA binding; cytoplasm; hydrolase activity; lipid metabolism; palmitoyl-CoA hydrolase activity; serine esterase activity
AA203329	0.000200052				
T89417	0.000205386				
R20209	0.000206661				
R41888	0.000209395				
R18261	0.000210355				
H65945	0.000214501				

R20223	0.000214646	ARHGDIA	396	Rho GDP dissociation inhibitor (GDI) alpha	GTPase activator activity; Rho GDP-dissociation inhibitor activity; Rho protein signal transduction; cytoplasm; negative regulation of cell adhesion; protein binding
AA099373	0.000217019	GYS1	2997	glycogen synthase 1 (muscle)	glycogen metabolism
H48578	0.000217829				
W17311	0.000218848	SDHB	6390	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	aerobic respiration; electron transport; electron transporter activity; mitochondrion; oxidoreductase activity; quinol:fumarate oxidoreductase activity; succinate dehydrogenase (ubiquinone) activity; tricarboxylic acid cycle
R34648	0.000219625	CHL1	10752	cell adhesion molecule with homology to L1CAM (close homolog of L1)	cell adhesion; integral to membrane; signal transduction
T83013	0.00022038	HGD	3081	homogentisate 1,2-dioxygenase (homogentisate oxidase)	
H62473	0.000221357	TGFBR3	7049	transforming growth factor, beta receptor III (betaglycan, 300kDa)	TGFbeta receptor signaling pathway; development; glycosaminoglycan binding; integral to membrane; receptor activity; signal transduction
H46382	0.000222325	PFTK1	5218	PFTAIRE protein kinase 1	ATP binding; cAMP-dependent protein kinase activity; cytoplasm; nucleus; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; transferase activity
N76007	0.000222498				
R31471	0.000229077				
AA034962	0.000230532				
R55936	0.000230896				
R00940	0.000233118				
R94499	0.000233405	GNB5	10681	guanine nucleotide binding protein (G protein), beta 5	

W51811	0.000236308	WNT5A	7474	wingless-type MMTV integration site family, member 5A	cell-cell signaling; development; embryogenesis and morphogenesis; extracellular space; frizzled-2 signaling pathway; receptor binding; signal transduction; soluble fraction
H77599	0.000241297				
W01319	0.000249061	BHC80	51317	BRAF35/HDAC2 complex (80 kDa)	
AA007532	0.000250771				
T95166	0.000251343				
AA026351	0.000251677				
R43244	0.000252089	PPOX	5498	protoporphyrinogen oxidase	electron transport; electron transporter activity; heme biosynthesis; mitochondrion; oxidoreductase activity; protoporphyrinogen oxidase activity
R21107	0.000252412				
R25521	0.000256979	NRCAM	4897	neuronal cell adhesion molecule	cell adhesion; cell adhesion molecule activity; integral to plasma membrane; tumor suppressor
H62909	0.000260347				
N23942	0.000269889	MEP50	79084	MEP50 protein	
H61379	0.000271804				
R83014	0.000275307				
H12528	0.000276615	ANXA5	308	annexin A5	anticoagulant activity; blood coagulation; calcium ion binding; calcium-dependent phospholipid binding; phospholipase inhibitor activity
R98591	0.000277306				
AA027815	0.000280858	KIAA1311	54439	KIAA1311 protein	nucleic acid binding
R24904	0.000283965	PP1665	81544	hypothetical protein PP1665	
R20383	0.000284443				

T92003	0.000286696	KIAA0342	9881	KIAA0342 gene product	DNA binding; membrane; nucleus; transport; transporter activity
H41889	0.000289679				
R45684	0.000291469				
H05447	0.000294637	SPG7	6687	spastic paraplegia 7, paraplegin (pure and complicated autosomal recessive)	ATP binding; cell adhesion; cell adhesion molecule activity; chaperone activity; extracellular matrix; integral to membrane; metalloendopeptidase activity; mitochondrion; neurogenesis; nucleotide binding; proteolysis and peptidolysis; signal transduction
H93445	0.000296991				
R13346	0.0003047	ENT4	222962	equilibrative nucleoside transporter 4	membrane; nucleoside transporter activity; transport
AA035446	0.000315977				
H77569	0.000318214				
AA203184	0.000320475				
R40860	0.000321443				
AA203714	0.000327318	G3BP2	9908	Ras-GTPase activating protein SH3 domain-binding protein 2	RAS protein signal transduction; RNA binding; cytoplasm; cytoplasmic sequestering of NF-kappaB; nucleus; protein transporter activity; protein-nucleus import; receptor signaling complex scaffold activity; transport
W47153	0.000339402	PTRF	284119	polymerase I and transcript release factor	
R98694	0.000341697	LOC286071	286071	hypothetical protein LOC286071	
T70299	0.000352597				

AA131933	0.00035343608	ABP1	26	amiloride binding protein 1 (amine oxidase (copper-containing))	amine oxidase (copper-containing) activity; copper ion binding; drug binding; heparin binding; metabolism; oxidoreductase activity; peroxisome
N22313	0.00035508	COL5A1	1289	collagen, type V, alpha 1	cell adhesion; collagen; collagen type V; extracellular matrix structural constituent; heparin binding
W32180	0.00036228 4				
N92610	0.00036306 5	ENPP5	59084	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)	hydrolase activity; nucleotide metabolism
AA059277	0.00036368 3				
R92201	0.00036531 4	LOC131118	131118	similar to RIKEN cDNA 1810055D05	
H86148	0.00036570 8				
R92085	0.00036705				
H93071	0.00037186 9				
H20128	0.00037654 3				
AA036798	0.00038025	LOC253827	253827	hypothetical protein LOC253827	
R35932	0.00038047 4				
R89790	0.00038193 2				
AA058463	0.00038437 2				
R35706	0.00038623 4	GPM6A	2823	glycoprotein M6A	integral to plasma membrane
R01927	0.00039130 2	FST	10468	follistatin	activin inhibitor activity; development; extracellular; negative regulation of follicle-stimulating hormone secretion

H03532	0.000391386	DLG5	9231	discs, large (Drosophila) homolog 5	cell growth and/or maintenance; cell-cell adhesion; intracellular signaling cascade; plasma membrane; protein binding; receptor signaling complex scaffold activity; regulation of cell cycle
R93705	0.000393695				
R00643	0.000396004				
R61879	0.000396078				
T80525	0.000399471				
H17380	0.000401317				
R93211	0.000408894				
R18947	0.000417953				
N49068	0.000419155	TMLHE	55217	trimethyllysine hydroxylase, epsilon	
R13038	0.000425346	LOC127262	127262	hypothetical protein LOC127262	
R46521	0.000429681	TAGLN2	8407	transgelin 2	muscle development
H16828	0.000446924	ARPP-19	10776	cyclic AMP phosphoprotein, 19 kD	cytoplasm; positive regulation of gluconeogenesis; positive regulation of glucose import; potassium channel regulator activity; receptor binding
H77738	0.000460239				
H84133	0.000464071	FLJ36040	162963	hypothetical protein FLJ36040	nucleic acid binding; nucleus; regulation of transcription, DNA-dependent
AA033714	0.000465544	FLJ14260	80095	hypothetical protein FLJ14260	DNA binding; metallopeptidase activity; nucleus; proteolysis and peptidolysis; regulation of transcription, DNA-dependent; zinc ion binding
N31736	0.000470422	TRIPIN	151246	tripin	
T91013	0.000473871				
H74032	0.00048042				

H75766	0.000480722	CR1	1378	complement component (3b/4b) receptor 1, including Knops blood group system	complement activation; complement component C3b receptor activity; complement receptor activity; integral to plasma membrane
W31293	0.000483427				
H01085	0.000483751				
W47003	0.000484083	HIF1A	3091	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	
R43963	0.000487097	DKFZp761A052	55593	hypothetical protein DKFZp761A052	
W85688	0.000493237				
R14617	0.000494827	EDRF1	26098	erythroid differentiation-related factor 1	
N24178	0.00051216				
AA026902	0.000521568	FLJ11320	55343	GDP-fucose transporter 1	Golgi apparatus; integral to membrane; sugar porter activity; transport
T89772	0.000528763	KIAA0767	23151	KIAA0767 protein	
H44375	0.000529467	RFXANK	8625	regulatory factor X-associated ankyrin-containing protein	humoral immune response; nucleus; regulation of transcription, DNA-dependent; transcription co-activator activity; transcription factor activity; transcription from Pol II promoter
H14566	0.000530146				
R88098	0.000530204	MYO1C	4641	myosin IC	ATP binding; actin binding; calmodulin binding; motor activity; perception of sound; unconventional myosin
R35559	0.000534118	PGR1	93621	T-cell activation protein	
R98556	0.000552901	CYB5	1528	cytochrome b-5	cytochrome-c oxidase activity; electron transport; energy pathways; integral to membrane; microsome; mitochondrion
R39119	0.000556				

	986				
R40156	0.000560126	ATSV	547	axonal transport of synaptic vesicles	ATP binding; anterograde axon cargo transport; kinesin complex; microtubule-based process; motor activity
T77530	0.000568511				
T95249	0.000586757	FMO3	2328	flavin containing monooxygenase 3	dimethylaniline monooxygenase (N-oxide-forming) activity; disulfide oxidoreductase activity; electron transport; integral to membrane; microsome
H02590	0.000592926				
R20809	0.0005996	RPLP1	6176	ribosomal protein, large, P1	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); intracellular; ribosome; structural constituent of ribosome; translational elongation
H06744	0.000600687				
W85877	0.000608409				
N29108	0.000611766				
H41330	0.000616443	LRRC2	79442	leucine-rich repeat-containing 2	
AA042920	0.000622675	GLI4	2738	GLI-Kruppel family member GLI4	DNA binding; biological_process unknown; molecular_function unknown; nucleus
W01227	0.000628914	HDGF	3068	hepatoma-derived growth factor (high-mobility group protein 1-like)	cell proliferation; cytoplasm; extracellular space; growth factor activity; heparin binding; signal transduction
R06054	0.000629027				
R22945	0.000630876	ALG2	85365	GDP-Man:Man(1)GlcNAc(2)-PP-dolichol mannosyltransferase	
AA101859	0.000631871	ENSA	2029	endosulfine alpha	ion channel inhibitor activity; receptor binding; response to nutrients; transport

R55497	0.000644459	MGC10485	112936	hypothetical protein MGC10485	cytosol; intracellular protein transport; protein binding; protein transporter activity; retrograde (endosome to Golgi) transport
H50657	0.000650395				
H73931	0.000654121				
R08735	0.000674376				
N71628	0.000686384	SPIB	6689	Spi-B transcription factor (Spi-1/PU.1 related)	RNA polymerase II transcription factor activity; biological_process unknown; cytoplasm; molecular_function unknown; nucleus; regulation of transcription from Pol II promoter; transcription factor activity
H95277	0.000692001		255065	LOC255065	
R25544	0.000714957	PCCB	5096	propionyl Coenzyme A carboxylase, beta polypeptide	fatty acid catabolism; mitochondrion; propionyl-CoA carboxylase activity
N/A1	0.000715432				
W15154	0.000717259	FLJ11712	79621	hypothetical protein FLJ11712	
R98517	0.000726742	HIST1H1C	3006	histone 1, H1c	DNA binding; chromosome; chromosome organization and biogenesis (sensu Eukarya); nucleosome; nucleosome assembly; nucleus
R48249	0.00072921		91170	hypothetical gene supported by AK002208	
N59242	0.000732796	HH114	84529	hypothetical protein HH114	
R11233	0.000734278	P2RX5	5026	purinergic receptor P2X, ligand-gated ion channel, 5	ATP binding; ion channel activity; ion transport; membrane; receptor activity
R06542	0.000735728				

R48990	0.000738059	NUP155	9631	nucleoporin 155kDa	nuclear pore; nucleocytoplasmic transport; nucleocytoplasmic transporter activity; nucleus; transport; transporter activity
R25101	0.000738646	NR2F2	7026	nuclear receptor subfamily 2, group F, member 2	ligand-regulated transcription factor activity; lipid metabolism; nucleus; regulation of transcription from Pol II promoter; signal transduction; steroid hormone receptor activity; transcription co-repressor activity; transcription factor activity
AA033764	0.000750967				
T94800	0.000755497				
AA033635	0.000762319				
H39673	0.000765384		352246	LOC352246	
AA031347	0.000765759				
R14520	0.000766904				
R69584	0.000776372				
N54947	0.000782204	GOLGA2	2801	golgi autoantigen, golgin subfamily a, 2	Golgi apparatus
R66251	0.000783046				
H37798	0.000785652				
R08249	0.000798521	KIAA0317	9870	KIAA0317 gene product	intracellular; ubiquitin cycle; ubiquitin-protein ligase activity
H84657	0.000810932	GRWD	83743	glutamate rich WD repeat protein GRWD	
W68275	0.000827038	CDC42EP2	10435	CDC42 effector protein (Rho GTPase binding) 2	
R43233	0.000832554		349228	hypothetical gene supported by AJ420560	
T70278	0.000837711				

H17081	0.000837798	COG7	91949	component of oligomeric golgi complex 7	Golgi apparatus; intracellular protein transport; membrane; protein transporter activity
W24394	0.000840149	A2M	2	alpha-2-macroglobulin	intracellular protein transport; protein carrier activity; serine protease inhibitor activity; wide-spectrum protease inhibitor activity
N32293	0.000841745	SEC24B	10427	SEC24 related gene family, member B (S. cerevisiae)	COPII vesicle; Golgi apparatus; endoplasmic reticulum; intracellular protein transport; membrane; protein transporter activity; secretory vesicle; vesicle-mediated transport
R56055	0.000856 06	RIC3	79608	RIC3 protein	
H49369	0.000860187	RAB3IL1	5866	RAB3A interacting protein (rabin3)-like 1	
W47177	0.000861608	GCN5L1	2647	GCN5 general control of amino-acid synthesis 5-like 1 (yeast)	biological_process unknown; cellular_component unknown; molecular_function unknown
H63763	0.000872181				
R07997	0.000876518				
H65775	0.000893253				
R19554	0.000910211				
N91376	0.000919833	KIAA0247	9766	KIAA0247 gene product	integral to membrane
AA031465	0.000923723	GEFT	115557	RAC/CDC42 exchange factor	
AA213450	0.000934614				
AA128013	0.000940491				
H40682	0.000941948				
R24850	0.000942	HSPA6	3310	heat shock	ATP binding; heat shock protein

	491			70kDa protein 6 (HSP70B')	activity
N25927	0.000950965				
R18453	0.000960994				
AA054541	0.000965547				
N52765	0.000966351	CACNA1I	8911	calcium channel, voltage-dependent, alpha 1I subunit	calcium ion binding; calcium ion transport; cation transport; integral to membrane; low voltage-gated calcium channel activity; voltage-gated calcium channel complex
H18581	0.000968418	CD164	8763	CD164 antigen, sialomucin	cell adhesion; cell adhesion molecule activity; development; immune response; integral to membrane; integral to plasma membrane; membrane fraction; negative regulation of cell proliferation; plasma membrane; signal transduction; soluble fraction
T79481	0.000972193	ANK1	286	ankyrin 1, erythrocytic	actin cytoskeleton; plasma membrane; structural constituent of cytoskeleton
N25754	0.000991313	LOC283378	283378	hypothetical protein LOC283378	
W03627	0.000992082	DELGEF	26297	deafness locus associated putative guanine nucleotide exchange factor	
H57509	0.000992872				
N31362	0.000995169	NF1	4763	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	GTPase activator activity; RAS protein signal transduction; Ras GTPase activator activity; cell growth and/or maintenance; cytoplasm; enzyme inhibitor activity; negative regulation of cell proliferation; tumor suppressor
H23343	0.000999695				
AA001336	0.001000548				
N77560	0.001011142				

W02503	0.001017699	SLC25A10	1468	solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10	binding; dicarboxylic acid transport; dicarboxylic acid transporter activity; gluconeogenesis; integral to membrane; mitochondrial inner membrane; mitochondrial transport; mitochondrion; transport
AA029583	0.001020959	TFF3	7033	trefoil factor 3 (intestinal)	defense response; digestion; extracellular
R68695	0.001026928				
W47411	0.001027216	THY1	7070	Thy-1 cell surface antigen	integral to plasma membrane
H69318	0.001039787	ADAM10	102	a disintegrin and metalloproteinase domain 10	cell-cell signaling; hydrolase activity; integral to plasma membrane; metalloendopeptidase activity; proteolysis and peptidolysis; zinc ion binding
H59247	0.001041911				
H61751	0.001043209	SLC5A2	6524	solute carrier family 5 (sodium/glucose cotransporter), member 2	carbohydrate metabolism; integral to membrane; low-affinity glucose:sodium symporter activity; sodium ion transport; sugar porter activity; symporter activity; transport; transporter activity
W90745	0.001045087				
R99293	0.001045597				
H13102	0.001050919				
R88720	0.001054557				
R55829	0.001056852	EDN1	1906	endothelin 1	cell-cell signaling; extracellular space; pathogenesis; peptide hormone; positive regulation of cell proliferation; regulation of blood pressure; signal transduction; soluble fraction; toxin activity
N25619	0.001063803				
AA035066	0.001077269	MGC4268	83607	hypothetical protein MGC4268	

AA001147	0.001079796	GK001	57003	GK001 protein	
R26320	0.001090599	FLNB	2317	filamin B, beta (actin binding protein 278)	actin binding; actin cytoskeleton; actin cytoskeleton organization and biogenesis; cytoskeletal anchoring; integral to plasma membrane; membrane associated actin binding; signal transduction
N24163	0.001097639				
AA136271	0.00109848				
T74280	0.001106704				
N64618	0.001111281	BUB3	9184	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)	cell proliferation; kinetochore; mitosis; mitotic spindle checkpoint; nucleus
R21092	0.001117965	CA1	759	carbonic anhydrase I	carbonate dehydratase activity; cytoplasm; lyase activity; one-carbon compound metabolism; zinc ion binding
R23778	0.001161765	C7	730	complement component 7	complement activation, alternative pathway; complement activation, classical pathway; complement activity; cytolysis; immune response; integral to membrane; membrane attack complex; response to pathogenic bacteria
AA142989	0.001176611	BMPER	168667	likely ortholog of mouse BMP-binding endothelial regulator precursor protein	calcium ion binding; extracellular
AA045611	0.001181504	FLJ20280	54876	hypothetical protein FLJ20280	
T87794	0.001190228				
H08517	0.001191761				

R47859	0.001191811	NPR1	4881	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	ATP binding; cGMP biosynthesis; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; peptide receptor activity, G-protein coupled; protein amino acid phosphorylation; protein kinase activity; receptor activity; receptor guanylate cyclase activity; regulation of blood pressure
R09247	0.001197335				
N34765	0.001201456				
W42881	0.001203458	ERdj5	54431	ER-resident protein ERdj5	
R16934	0.001203671	TNFSF13B	10673	tumor necrosis factor (ligand) superfamily, member 13b	cell proliferation; immune response; integral to plasma membrane; positive regulation of cell proliferation; signal transduction; soluble fraction; tumor necrosis factor receptor binding
AA044940	0.001204383				
R56037	0.001231967				
AA040160	0.001246704	LOC92715	92715	hypothetical protein BC017335	
R83852	0.001251331				
R13550	0.001254784				
H26570	0.001277725	AKT2	208	v-akt murine thymoma viral oncogene homolog 2	ATP binding; protein amino acid phosphorylation; protein serine/threonine kinase activity; transferase activity
N63396	0.001314843				
H51271	0.001320774				
AA004845	0.001331715	KIAA1529	57653	KIAA1529 protein	
N/A1	0.001335105				
W07648	0.001344377				
T66873	0.001346				

	468				
R14326	0.001349613	HERC1	8925	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1	ARF guanyl-nucleotide exchange factor activity; Golgi apparatus; catalytic activity; nonselective vesicle transport; ubiquitin cycle; ubiquitin-protein ligase activity
H98668	0.001354685				
AA002135	0.001365198	C2	717	complement component 2	chymotrypsin activity; classical-complement-pathway C3/C5 convertase activity; complement activation, classical pathway; complement component C2 complex; hydrolase activity; proteolysis and peptidolysis; trypsin activity
R12665	0.001368855		197135	similar to RIKEN cDNA 4930424G05	
N38953	0.001373325				
N90841	0.001376131	CDKN1C	1028	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	G1 phase of mitotic cell cycle; cell cycle; cell cycle arrest; cyclin-dependent protein kinase inhibitor activity; negative regulation of cell cycle; negative regulation of cell proliferation; nucleus; regulation of CDK activity
R42536	0.00137895	DACH	1602	dachshund homolog (Drosophila)	eye morphogenesis (sensu Drosophila)
R34537	0.001381264				
R43469	0.001381555	EPHB3	2049	EphB3	ATP binding; ephrin receptor activity; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; signal transduction; transferase activity; transmembrane receptor protein tyrosine kinase signaling pathway
T78084	0.001391293				
T86217	0.001431738				

R09469	0.001432031				
H67999	0.001432285	CYP3A7	1551	cytochrome P450, family 3, subfamily A, polypeptide 7	cytochrome P450 activity
T95687	0.001435574	RAP1GDS1	5910	RAP1, GTP-GDP dissociation stimulator 1	GTPase activator activity; biological_process unknown; cellular_component unknown
H64569	0.001442673				
R09844	0.001443567				
N49914	0.001454344	KIAA0423	23116	KIAA0423 protein	binding; mitochondrial inner membrane; transport
W80729	0.001458212	SMUG1	23583	single-strand selective monofunctional uracil DNA glycosylase	DNA repair; single-stranded DNA binding; uracil DNA N-glycosylase activity
R06074	0.001462305				
AA031859	0.001463558	TIMM13	26517	translocase of inner mitochondrial membrane 13 homolog (yeast)	hearing; mitochondrial inner membrane pre-sequence translocase complex; mitochondrial translocation; mitochondrion; protein targeting; protein translocase activity; zinc ion binding
AA034109	0.001468252	MINK	50488	misshapen/NIK-related kinase	ATP binding; cAMP-dependent protein kinase activity; development; protein amino acid phosphorylation; protein kinase CK2 activity; protein kinase cascade; protein serine/threonine kinase activity; response to stress; small GTPase regulatory/interacting protein activity; transferase activity
R34114	0.001469248				
R23880	0.001488865		340730	LOC340730	

R09962	0.001493614	PMS2L6	5384	postmeiotic segregation increased 2-like 6	damaged DNA binding; mismatch repair; nucleus
H82747	0.001495072				
R20140	0.001495222	MFN1	55669	mitofusin 1	
T84619	0.001499292				
T95148	0.001503153				
R18138	0.001512918				
H01884	0.001532617				
W90154	0.001537062	DKFZP434B168	25896	DKFZP434B168 protein	
R06564	0.001538843	GALE	2582	galactose-4-epimerase, UDP-	UDP-glucose 4-epimerase activity; carbohydrate metabolism; galactose metabolism; isomerase activity; nucleotide-sugar metabolism
N54603	0.001548733				
AA039986	0.001567752	FBLP-1	54751	filamin-binding LIM protein-1	
N21636	0.001573222	PBP	5037	prostatic binding protein	ATP binding; lipid binding; phosphatidylethanolamine binding; serine protease inhibitor activity
H08101	0.001597637	GA	27165	liver mitochondrial glutaminase	amino acid metabolism; glutaminase activity; glutamine metabolism; hydrolase activity; mitochondrion
N49325	0.001617303	KIAA0962	23341	KIAA0962 protein	
W69432	0.001627691	MAPKAPK2	9261	mitogen-activated protein kinase-activated protein kinase 2	ATP binding; MAPKKK cascade; nucleus; protein amino acid phosphorylation; protein serine/threonine kinase activity; signal transducer activity; transferase activity
AA031630	0.001635462	LOC340061	340061	hypothetical protein LOC340061	
R97368	0.001637				

	009				
H67706	0.001639338				
R36169	0.00164831				
AA135646	0.001668986	hIAN6	155038	human immune associated nucleotide 6	
T79552	0.001673764				
T67217	0.001676026	MGC3207	84245	hypothetical protein MGC3207	
R65751	0.001696666	SLC16A4	9122	solute carrier family 16 (monocarboxylic acid transporters), member 4	integral to plasma membrane; membrane fraction; monocarboxylic acid transport; monocarboxylic acid transporter activity
AA033652	0.001697509	DDX54	79039	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54	
R44335	0.00170669				
W03006	0.001708944	MARCKS	4082	myristoylated alanine-rich protein kinase C substrate	actin cross-linking activity; actin cytoskeleton; calmodulin binding; cell motility; plasma membrane
R56247	0.00171031	RASD2	23551	RASD family, member 2	GTP binding; RAS small monomeric GTPase activity; biological_process unknown; cellular_component unknown; molecular_function unknown; small GTPase mediated signal transduction
R76088	0.001718762	UCHL3	7347	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)	cytoplasm; hydrolase activity; ubiquitin thiolesterase activity; ubiquitin-dependent protein catabolism
T84202	0.001725688	TAPBP	6892	TAP binding protein (tapasin)	MHC-interacting protein; endoplasmic reticulum; endoplasmic reticulum membrane; immune response; integral to membrane; peptide antigen transporter activity; protein binding; protein complex assembly

N23779	0.001736785	CD151	977	CD151 antigen	cell adhesion; integral to plasma membrane; membrane fraction
R28465	0.001739327		221922	hypothetical gene supported by AL713633; BC014395	
R94942	0.001743843	FLJ20522	54965	hypothetical protein FLJ20522	
R17806	0.001749212	C14orf37	145407	chromosome 14 open reading frame 37	
T99284	0.001774889		145608	LOC145608	
T97033	0.001780573	DKFZP434I116	25962	DKFZP434I116 protein	nucleic acid binding
H98614	0.001790742	ZFHX1B	9839	zinc finger homeobox 1b	SMAD binding; negative regulation of transcription; neurogenesis; nucleus; phosphatase regulator activity; regulation of transcription, DNA-dependent; transcription factor activity; transcriptional repressor activity
R07557	0.001802913	RPLP0	6175	ribosomal protein, large, P0	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); intracellular; ribosome; structural constituent of ribosome; translational elongation
H59829	0.001815986				
T85888	0.001824002	DKFZP564O043	57037	hypothetical protein DKFZp564O043	
R80523	0.001824324				
T70331	0.001825185	EPAS1	2034	endothelial PAS domain protein 1	RNA polymerase II transcription factor activity, enhancer binding; angiogenesis; development; nucleus; regulation of transcription, DNA-dependent; signal transducer activity; signal transduction; transcription co-activator activity; transcription from Pol II promoter
H77595	0.001827873				
R69535	0.001829561	IGKC	3514	immunoglobulin kappa constant	antigen binding; immune response
R13273	0.001843677				
T72401	0.001845	C8orf4	56892	chromoso	

	907			me 8 open reading frame 4	
H47114	0.00184768				
T99834	0.00186704 9				
H68599	0.00186986 6	MGC15737	85012	hypothetical protein MGC15737	
N57396	0.00189057 2	LOC150837	150837	hypothetical protein LOC150837	
R23436	0.00190003	PRKAR2A	5576	protein kinase, cAMP-dependent, regulatory, type II, alpha	cAMP-dependent protein kinase, intrinsic regulator activity; cytoplasm; intracellular signaling cascade; membrane fraction; plasma membrane
R21825	0.00191534 5				
AA114919	0.00191781 5	NSEP1	4904	nuclease sensitive element binding protein 1	DNA binding; double-stranded DNA binding; nucleus; response to pest/pathogen/parasite; single-stranded DNA binding; transcription factor activity; transcription from Pol II promoter
R02194	0.00192130 2				
N24943	0.00193564 1	FLJ13612	80303	likely ortholog of neuronally expressed calcium binding protein	calcium ion binding
R87060	0.00195250 1	GGCX	2677	gamma-glutamyl carboxylase	blood coagulation; gamma-glutamyl carboxylase activity; integral to membrane; ligase activity; membrane fraction; protein modification
AA045253	0.00196595 1				
R00170	0.00196931 6	BFAR	51283	bifunctional apoptosis regulator	anti-apoptosis; apoptosis inhibitor activity; integral to plasma membrane; membrane fraction; structural molecule activity
R63553	0.00197175 2	ALEX1	51309	ALEX1 protein	
T82206	0.00198511 6				

H17654	0.001985446				
T65080	0.001987878				
H85905	0.002010733				
R80226	0.002037069				
AA057286	0.002040228	TA-WDRP	134430	T-cell activation WD repeat protein	catalytic activity; metabolism
N59347	0.002086366	TARS	6897	threonyl-tRNA synthetase	ATP binding; cytoplasm; ligase activity; soluble fraction; threonine-tRNA ligase activity; threonyl-tRNA aminoacylation
R12879	0.002091929	KIAA1336	57539	KIAA1336 protein	
H42536	0.002097603	GPD1	2819	glycerol-3-phosphate dehydrogenase 1 (soluble)	carbohydrate metabolism; glycerol-3-phosphate dehydrogenase (NAD) activity; glycerol-3-phosphate dehydrogenase complex; glycerol-3-phosphate metabolism; oxidoreductase activity, acting on CH-OH group of donors
R20019	0.00211861				
AA207094	0.002132152				
H84720	0.002135212				
N67634	0.002150875	P1P373C6	56053	hypothetical protein P1 p373c6	
R36086	0.002150941				
N64399	0.002151364	OSBPL1A	114876	oxysterol binding protein-like 1A	cholesterol metabolism; intracellular; lipid transport; phospholipid binding; steroid metabolism; vesicle-mediated transport
AA203254	0.002155188				
N76908	0.002175852				
R31105	0.002197489				
T81437	0.002224647				

N75416	0.002233007	GNA14	9630	guanine nucleotide binding protein (G protein), alpha 14	G-protein coupled receptor protein signaling pathway; GTP binding; heterotrimeric G-protein GTPase activity; heterotrimeric G-protein complex; plasma membrane; protein amino acid ADP-ribosylation; signal transducer activity; signal transduction
T75185	0.002242925	FLJ33761	125488	hypothetical protein FLJ33761	
AA150729	0.002252663	NCOR2	9612	nuclear receptor co-repressor 2	
AA203692	0.002254753				
R15267	0.002276617				
AA046245	0.00228188	OSF-2	10631	osteoblast specific factor 2 (fasciclin I-like)	cell adhesion; cell adhesion molecule activity; extracellular matrix; skeletal development
R60906	0.002297729	MGC24381	115939	hypothetical protein MGC24381	
H59112	0.002297904				
N24066	0.002299348	CSDA	8531	cold shock domain protein A	RNA polymerase II transcription factor activity; cytoplasm; double-stranded DNA binding; negative regulation of transcription from Pol II promoter; perinuclear space; response to cold; transcription co-repressor activity; transcription factor activity
H80679	0.002311983				
N78909	0.00231535				
N/A1	0.002315428				
W47145	0.002340933	EIF3S7	8664	eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa	eukaryotic translation initiation factor 3 complex; protein biosynthesis; regulation of translational initiation; translation initiation factor activity
N62985	0.002361781	LOC90673	90673	hypothetical protein LOC90673	

N42004	0.002367601	DPYSL3	1809	dihydropyrimidinase-like 3	dihydropyrimidinase activity; hydrolase activity; neurogenesis; nucleobase, nucleoside, nucleotide and nucleic acid metabolism; signal transduction
R65766	0.002368414	SEC22L1	9554	SEC22 vesicle trafficking protein-like 1 (S. cerevisiae)	ER to Golgi transport; endoplasmic reticulum membrane
R36114	0.002399498	FLJ33387	161145	hypothetical protein FLJ33387	
H85811	0.002403043	HIPK2	28996	homeodomain interacting protein kinase 2	nucleus; protein kinase activity; transcription co-repressor activity
AA130140	0.002403115				
T93785	0.002410919				
H62006	0.002432551	EVI2B	2124	ecotropic viral integration site 2B	cell growth and/or maintenance; integral to plasma membrane
R25519	0.002434119	HPCAL4	51440	hippocalcin like 4	calcium ion binding; central nervous system development
H57272	0.002474056				
R09933	0.002511822				
W15390	0.002545125	BMPR1A	657	bone morphogenetic protein receptor, type IA	ATP binding; TGFbeta receptor signaling pathway; cAMP-dependent protein kinase activity; integral to membrane; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; protein-tyrosine kinase activity; receptor activity; transferase activity; transforming growth factor-beta receptor activity
R16440	0.002546399	AD024	57405	AD024 protein	
AA010141	0.002547572	SERPINH1	871	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47),	heat shock response

				member 1, (collagen binding protein 1)	
R88895	0.002561656	MANBAL	63905	mannosidase, beta A, lysosomal-like	integral to membrane
H84599	0.00258041				
H06620	0.002605411				
H79410	0.002607092				
H19945	0.002611532	STX5A	6811	syntaxin 5A	nonselective vesicle targeting
AA142924	0.00261233	DF	1675	D component of complement (adipsin)	chymotrypsin activity; complement activation, alternative pathway; complement factor D activity; hydrolase activity; proteolysis and peptidolysis; trypsin activity
W39594	0.002627393	NSEP1	4904	nuclease sensitive element binding protein 1	DNA binding; double-stranded DNA binding; nucleus; response to pest/pathogen/parasite; single-stranded DNA binding; transcription factor activity; transcription from Pol II promoter
R10547	0.002656444				
H69845	0.002659182				
AA031564	0.002660207	LOC113444	113444	hypothetical protein BC011880	
AA152287	0.002679818	SLC35B2	347734	solute carrier family 35, member B2	copper ion binding; electron transport; electron transporter activity
AA028109	0.002693002	RAB23	51715	RAB23, member RAS oncogene family	GTP binding; RAB small monomeric GTPase activity; intracellular protein transport; protein transporter activity; small GTPase mediated signal transduction
T91181	0.002737341				

AA054102	0.00274309 1	SOCS5	9655	suppressor of cytokine signaling 5	JAK-STAT cascade; cytokine and chemokine mediated signaling pathway; cytoplasm; intracellular signaling cascade; kinase inhibitor activity; negative regulation of T-helper 2 cell differentiation; negative regulation of signal transduction; positive regulation of T-helper 1 cell differentiation; protein binding; regulation of cell growth
N94432	0.00274606 8				
H28534	0.00278444 4	AQP1	358	aquaporin 1 (channel-forming integral protein, 28kDa)	excretion; integral to plasma membrane; transport; water transport; water transporter activity
N90527	0.00279075	PIM1	5292	pim-1 oncogene	ATP binding; cAMP-dependent protein kinase activity; cell growth and/or maintenance; cytoplasm; development; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; transferase activity
AA114872	0.00280135 3	ACO1	48	aconitase 1, soluble	RNA binding; aconitate hydratase activity; cytoplasm; lyase activity; metabolism; negative regulation of translation; tricarboxylic acid cycle
AA046610	0.00280435 8				
R88547	0.00282404 7	FLJ25530	220296	hypothetical protein FLJ25530	
N24815	0.00284845 3	UBA52	7311	ubiquitin A-52 residue ribosomal protein fusion product 1	nucleus; protein biosynthesis; protein modification; ribosome; structural constituent of ribosome
R52303	0.00284958 3				
N45514	0.00287018 6	NECL1	57863	nectin-like protein 1	
N75085	0.00288008 2	OLR1	4973	oxidised low density lipoprotein (lectin-like) receptor 1	circulation; heterophilic cell adhesion; integral to plasma membrane; membrane fraction; proteolysis and peptidolysis; receptor activity; sugar binding
H53599	0.00289915 7				
H51648	0.002907	MGC1694	112479	similar to	exonuclease activity; intracellular

	82	3		RIKEN cDNA 4933424N09 gene	
N69468	0.002945715				
H82917	0.002960524				
R26716	0.002964644	ZBTB2	57621	zinc finger and BTB domain containing 2	DNA binding; nucleus; protein binding; regulation of transcription, DNA-dependent
N22392	0.002974381	CLDN11	5010	claudin 11 (oligodendrocyte transmembrane protein)	integral to membrane; structural molecule activity; tight junction
AA054115	0.002987791				
H88417	0.002994186	CGI-127	51646	yippee protein	
H72512	0.003003414	HSPC023	28974	HSPC023 protein	
T91277	0.00302545				
AA062622	0.003033254	PRKWNK1	65125	protein kinase, lysine deficient 1	
W32940	0.003035311	FLJ32115	121506	hypothetical protein FLJ32115	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen
R50905	0.003042081	TUBB	7280	tubulin, beta polypeptide	cytoskeleton; structural constituent of cytoskeleton
W47505	0.003050809	IGFBP5	3488	insulin-like growth factor binding protein 5	extracellular space; insulin-like growth factor binding; regulation of cell growth; signal transduction
T84813	0.003051414	VDAC2	7417	voltage-dependent anion channel 2	anion transport; integral to membrane; mitochondrial outer membrane; mitochondrion; voltage-dependent anion channel porin activity; voltage-dependent ion-selective channel activity
AA054746	0.003052362				

W90748	0.003061153				
W90109	0.003070945				
N39407	0.003085277	KIF21A	55605	kinesin family member 21A	
AA059211	0.003121245	MAK	4117	male germ cell-associated kinase	ATP binding; protein amino acid phosphorylation; protein serine/threonine kinase activity; spermatogenesis; transferase activity
AA059211	0.003121245		283963	hypothetical gene supported by AK094432	
H81468	0.003121494				
T85314	0.003146725				
T90080	0.003207004	SPAG9	9043	sperm associated antigen 9	integral to membrane; spermatogenesis
H00627	0.00322867				
H52273	0.003234343				
W60305	0.003247636				
T85676	0.00327422	VIL1	7429	villin 1	F-actin capping protein complex; actin binding; actin bundling activity; actin filament severing activity; protein complex assembly
N79080	0.003276459	PTMA	5757	prothymosin, alpha (gene sequence 28)	development; nucleus; regulation of cell cycle; transcription
R80424	0.003287749				
R91375	0.00332147				
T92329	0.003345347				
N95805	0.003352305	KIAA1284	27152	KIAA1284 protein	intracellular signaling cascade
N77126	0.003361642				
W58007	0.003380131		339299	LOC339299	

N75083	0.003398293	MMP15	4324	matrix metalloproteinase 15 (membrane-inserted)	enzyme activator activity; extracellular matrix; hydrolase activity; integral to plasma membrane; metalloendopeptidase activity; protein modification; proteolysis and peptidolysis; zinc ion binding
R87413	0.003431986	SEMA3B	7869	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	axon guidance; cell-cell signaling; endoplasmic reticulum
AA031958	0.003477242				
R75598	0.003519312	NBL1	4681	neuroblastoma, suppression of tumorigenicity 1	negative regulation of cell cycle
AA010093	0.003548005				
H44869	0.003579353				
W72400	0.003625373	C12orf2	11228	chromosome 12 open reading frame 2	neuropeptide signaling pathway
H68885	0.003639707	TSSC3	7262	tumor suppressing subtransferable candidate 3	apoptosis; imprinting
R37412	0.003648196	GSTT1	2952	glutathione S-transferase theta 1	glutathione transferase activity; response to stress; transferase activity
R26131	0.00366188	C6orf37	55603	chromosome 6 open reading frame 37	
R09692	0.003663018				

H24259	0.003672244	KIAA1010	23268	KIAA1010 protein	endocytosis; guanyl-nucleotide exchange factor activity; intracellular signaling cascade
R00688	0.003689215				
R22967	0.003710451	STAB1	23166	stabilin 1	
N55283	0.003739354	KIAA0469	9903	KIAA0469 gene product	
H99202	0.003764722	MGC4126	84859	hypothetical protein MGC4126	
AA129727	0.00378033	RAB5C	5878	RAB5C, member RAS oncogene family	GTP binding; RAB small monomeric GTPase activity; intracellular protein transport; protein transporter activity; small GTPase mediated signal transduction
AA136708	0.003781094				
R86045	0.003820446				
R15278	0.003834693	EIF2S2	8894	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa	RNA binding; eukaryotic translation initiation factor 2 complex; ribosome; translation initiation factor activity; translational initiation
H39156	0.003865716	MTMR6	9107	myotubularin related protein 6	cellular_component unknown; hydrolase activity; protein amino acid dephosphorylation; protein serine/threonine phosphatase activity; protein tyrosine phosphatase activity
H09744	0.003883417				
R46328	0.003890309	TNRC5	10695	trinucleotide repeat containing 5	
H63443	0.003904024				
W56823	0.003927879	FURIN	5045	furin (paired basic amino acid cleaving enzyme)	Golgi apparatus; cell-cell signaling; furin activity; hydrolase activity; integral to membrane; proteolysis and peptidolysis; subtilase activity
H66020	0.003968552	PIPOX	51268	pipecolic acid oxidase	oxidoreductase activity; peroxisome; sarcosine oxidase activity; tetrahydrofolate metabolism

R13974	0.003986489				
H50984	0.003986736				
T86807	0.003987072	STK19	8859	serine/threonine kinase 19	ATP binding; cAMP-dependent protein kinase activity; manganese ion binding; nucleus; protein amino acid phosphorylation; protein kinase CK2 activity; protein serine/threonine kinase activity; transferase activity
T86807	0.003987072				
AA150837	0.003991927				
H27730	0.004008584	PPP2R1B	5519	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	protein phosphatase type 2A, intrinsic regulator activity
H88208	0.004022824	SUPV3L1	6832	suppressor of var1, 3-like 1 (S. cerevisiae)	ATP binding; ATP dependent helicase activity; RNA binding; hydrolase activity; mitochondrion
R42763	0.004059641	KIAA0319	9856	KIAA0319 gene product	
H21697	0.00410295	TEGT	7009	testis enhanced gene transcript (BAX inhibitor 1)	apoptosis; endoplasmic reticulum; insoluble fraction; integral to plasma membrane; nucleus
N69188	0.004119935				
H65832	0.004144741				
AA029842	0.004147955	MTCP1	4515	mature T-cell proliferation 1	cell proliferation; oncogenesis; regulation of cell cycle
H01149	0.004149361	INPP5D	3635	inositol polyphosphate-5-phosphatase, 145kDa	inositol-polyphosphate 5-phosphatase activity; phosphate metabolism; signal transduction

AA128101	0.004162313	GM2A	2760	GM2 ganglioside activator protein	glycolipid catabolism; glycosphingolipid metabolism; lysosome; sphingolipid activator protein activity; sphingolipid catabolism
R97814	0.004168698	NACA	4666	nascent-polypeptide-associated complex alpha polypeptide	nascent polypeptide association; nascent polypeptide-associated complex; protein biosynthesis
AA015841	0.004170424	GNGT1	2792	guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1	G-protein coupled receptor protein signaling pathway; heterotrimeric G-protein GTPase activity; heterotrimeric G-protein complex; signal transducer activity; signal transduction
H82982	0.004178218	ZNF275	10838	zinc finger protein 275	DNA binding; nucleus; regulation of transcription, DNA-dependent
R59367	0.004178499				
R08181	0.00423452				
W03107	0.004235497				
R11934	0.00424795				
H95669	0.004256235				
N71659	0.004258619				
AA210692	0.004283972	KIAA0116	23016	KIAA0116 protein	3'-5' exoribonuclease activity; RNA binding; RNA catabolism; exonuclease activity; exosome (RNase complex); hydrolase activity; nucleus; rRNA processing
H75643	0.004302059				
R13675	0.00431009	PAK6	56924	p21(CDKN1A)-activated kinase 6	ATP binding; protein amino acid phosphorylation; protein serine/threonine kinase activity; protein-tyrosine kinase activity; transferase activity
N22152	0.004328295	LOC255743	255743	hypothetical protein LOC25574	

				3	
H78933	0.004339196	UAP1	6675	UDP-N-acteylglucosamine pyrophosphorylase 1	UDP-N-acetylglucosamine biosynthesis; UDP-N-acetylglucosamine diphosphorylase activity; metabolism; transferase activity
N98333	0.004367397	RPL7	6129	ribosomal protein L7	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; structural constituent of ribosome; transcription regulator activity
H60498	0.004370662				
N24030	0.004380518	IKBKG	8517	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	NIK-I-kappaB/NF-kappaB cascade; immune response; induction of apoptosis; kinesin complex; nucleus; regulation of transcription, DNA-dependent; signal transducer activity
N22335	0.004396797	LOC129531	129531	hypothetical protein BC018453	
T70828	0.00440295				
H28350	0.004422595				
R84287	0.004464223				
H67348	0.004474096	DKFZp761B128	144348	hypothetical protein DKFZp761B128	nucleus
W60936	0.00449298	TRIM8	81603	tripartite motif-containing 8	biological_process unknown; cellular_component unknown; kinesin complex; molecular_function unknown; nucleus; zinc ion binding
AA151307	0.004494561	GNB2	2783	guanine nucleotide binding protein (G protein), beta polypeptide 2	G-protein coupled receptor protein signaling pathway; heterotrimeric G-protein GTPase activity; heterotrimeric G-protein complex; signal transducer activity; signal transduction
W03758	0.004506816				
W58007	0.004509317		339299	LOC339299	

AA149298	0.004511158	DF	1675	D component of complement (adipsin)	chymotrypsin activity; complement activation, alternative pathway; complement factor D activity; hydrolase activity; proteolysis and peptidolysis; trypsin activity
AA021554	0.004538873	NRL	4901	neural retina leucine zipper	DNA binding; nucleus; regulation of rhodopsin gene activity; regulation of transcription, DNA-dependent; specific RNA polymerase II transcription factor activity; transcription from Pol II promoter; vision
N/A1	0.004554496				
AA011554	0.004565064				
N52439	0.004596897	KIDINS220	57498	likely homolog of rat kinase D-interacting substance of 220 kDa	
H45907	0.004617352	PKD1-like	79932	polycystic kidney disease 1-like	
H73375	0.004628302				
H61842	0.004643509				
W86566	0.004663212				
T83293	0.004663354				
R70369	0.004673353	GPX4	2879	glutathione peroxidase 4 (phospholipid hydroperoxidase)	development; electron transporter activity; glutathione peroxidase activity; mitochondrion; oxidoreductase activity; phospholipid metabolism; response to oxidative stress
W93335	0.00467543				
N48735	0.004677987				
R12985	0.004679098	SGNE1	6447	secretory granule, neuroendocrine protein 1 (7B2 protein)	GTP binding; enzyme activator activity; neuropeptide signaling pathway; secretory vesicle
R86861	0.00469402	RPIP8	10900	RaP2 interacting protein 8	small GTPase mediated signal transduction; small GTPase regulatory/interacting protein activity
R89284	0.004700856				

R53914	0.004722548	HARC	55664	Hsp90-associating relative of Cdc37	cytokinesis; regulation of cell cycle
H54108	0.004736708				
N29429	0.004746315	CGI-57	27013	hypothetical protein CGI-57	
AA028961	0.004756591				
H09429	0.004758092				
R97802	0.004761377				
R99685	0.004818624				
R96651	0.004819469	ATOX1	475	ATX1 antioxidant protein 1 homolog (yeast)	chaperone activity; copper ion binding; copper ion homeostasis; copper ion transport; metal ion binding; metal ion transport; response to oxidative stress
W04610	0.004828823	H3F3A	3020	H3 histone, family 3A	
R13333	0.004845099	LOC283445	283445	hypothetical protein LOC283445	
W15573	0.004884151	FLJ33957	121551	hypothetical protein FLJ33957	protein binding
AA128301	0.004889001				
AA147589	0.004889486				
H67736	0.004915864	PPY2	23614	pancreatic polypeptide 2	
AA037107	0.004945451	TGFA	7039	transforming growth factor, alpha	
W23575	0.004953754	PFKP	5214	phosphofructokinase, platelet	6-phosphofructokinase activity; 6-phosphofructokinase complex; glycolysis; kinase activity; magnesium ion binding; transferase activity
H21773	0.004960414	LOC145758	145758	hypothetical protein LOC145758	
R32813	0.004961057	MGC2668	81605	hypothetical protein	

				MGC2668	
H06538	0.004987676	KCNK9	51305	potassium channel, subfamily K, member 9	integral to membrane; ion transport; membrane fraction; potassium channel activity; potassium ion transport; voltage-gated ion channel activity
W63762	0.004989318	COX15	1355	COX15 homolog, cytochrome c oxidase assembly protein (yeast)	cytochrome-c oxidase activity; electron transporter activity; mitochondrion; respiratory gaseous exchange
N66115	0.005016971				
W52156	0.005026981	OXTR	5021	oxytocin receptor	G-protein signaling, coupled to IP3 second messenger (phospholipase C activating); endosome; integral to plasma membrane; lactation; muscle contraction; oxytocin receptor activity; pregnancy; rhodopsin-like receptor activity; vasopressin receptor activity
AA054715	0.005032123				
W38730	0.005061733				
R88987	0.005075735	TTR	7276	transthyretin (prealbumin, amyloidosis type I)	carrier activity; extracellular space; retinol binding; steroid binding; thyroid hormone generation; thyroid hormone transporter activity; transport
T66929	0.005081981	FLJ21603	79818	hypothetical protein FLJ21603	
W05496	0.005082158	SEMA3F	6405	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F	development; extracellular space
H68528	0.005143893	FLJ32499	124637	hypothetical protein FLJ32499	
R18381	0.005146441				
H62158	0.005151				

	847				
H65385	0.005214822				
AA125808	0.005222531	CAPS	828	calcyphosine	calcium ion binding; intracellular signaling cascade
H51675	0.005230746				
T80134	0.005239732	TBCD	6904	tubulin-specific chaperoned	beta-tubulin folding; co-chaperonin activity; cytosol; microtubule; protein folding
W69323	0.00525725				
AA204664	0.005257297	SMC1L2	27127	SMC1 structural maintenance of chromosomes 1-like 2 (yeast)	ATP binding; ATP-binding cassette (ABC) transporter activity; cell cycle; chromosome segregation; kinesin complex; meiosis; membrane; nucleus; transport
H73896	0.005259852				
R87739	0.005263108				
H68720	0.005300974				
AA203318	0.005318056				
R99774	0.00531842	NT5C2	22978	5'-nucleotidase, cytosolic II	IMP-GMP specific 5'-nucleotidase activity; cytosol; hydrolase activity
R19118	0.005375804	SDCBP	6386	syndecan binding protein (syntenin)	actin modulating activity; adherens junction; cytoskeletal adaptor activity; cytoskeleton; endoplasmic reticulum; interleukin-5 receptor binding; interleukin-5 receptor complex; intracellular signaling cascade; membrane; neurexin binding; nucleus; protein-membrane targeting; regulation of synapse; substrate-bound cell migration, cell extension; syndecan binding
N62188	0.005378088				

N62259	0.005422106	DUSP1	1843	dual specificity phosphatase 1	CTD phosphatase activity; MAP kinase phosphatase activity; calcium-dependent protein serine/threonine phosphatase activity; cell cycle; hydrolase activity; magnesium-dependent protein serine/threonine phosphatase activity; myosin phosphatase activity; non-membrane spanning protein tyrosine phosphatase activity; protein amino acid dephosphorylation; protein phosphatase type 2A activity; protein phosphatase type 2B activity; protein phosphatase type 2C activity; response to oxidative stress
H48502	0.005425544	LOC339448	339448	hypothetical protein LOC339448	
H58461	0.005467797		339088	similar to My016 protein	
AA009926	0.005503977				
R34574	0.005535538				
H85857	0.005575674	LOC284352	284352	hypothetical protein LOC284352	
R34347	0.005611858	KIAA0354	9925	KIAA0354 gene product	protein binding
H46137	0.005658161	NAPB	63908	N-ethylmaleimide-sensitive factor attachment protein, beta	Golgi apparatus; endoplasmic reticulum; intracellular protein transport; intracellular transporter activity; protein transporter activity
T86459	0.005660196				
H70009	0.00569246				
N91458	0.005694363	TA-WDRP	134430	T-cell activation WD repeat protein	catalytic activity; metabolism
N30845	0.005715041	HPSE	10855	heparanase	beta-glucuronidase activity; inflammatory response; invasive growth; proteoglycan metabolism
R65850	0.005722				

	801				
N38966	0.005736658				
N34901	0.005737532	GALNT7	117248	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7	
W70144	0.005776662	VDR	7421	vitamin D (1,25-dihydroxyvitamin D3) receptor	nucleus; regulation of transcription, DNA-dependent; signal transduction; steroid hormone receptor activity; transcription factor activity; vitamin D3 receptor activity
H94763	0.005782828	SH3GLB1	51100	SH3-domain GRB2-like endophilin B1	
AA132089	0.005795396	FLJ20522	54965	hypothetical protein FLJ20522	
AA037207	0.005804992	MIRAB13	85377	molecule interacting with Rab13	GTPase regulator activity; intracellular; vesicle-mediated transport; zinc ion binding
AA098865	0.005823667	BCL2L10	10017	BCL2-like 10 (apoptosis facilitator)	anti-apoptosis; apoptosis inhibitor activity; caspase activation; female gamete generation; integral to membrane; membrane fraction; mitochondrion; protein binding; spermatogenesis
AA211819	0.005825701	MGC3130	78995	hypothetical protein MGC3130	
R83247	0.005844822	GLB1	2720	galactosidase, beta 1	beta-galactosidase activity; lysosome
T95864	0.005905684				
R10875	0.005906909	HSD17B1	3292	hydroxysteroid (17-beta) dehydrogenase 1	catalytic activity; cytoplasm; estrogen metabolism; steroid biosynthesis
R80475	0.005911476	FGFR1	2260	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	FGF receptor signaling pathway; MAPKKK cascade; fibroblast growth factor receptor activity; integral to plasma membrane; oncogenesis; skeletal development
H81331	0.005935414				

R54877	0.005962813	FLJ10415	55139	hypothetical protein FLJ10415	
R14363	0.005963375	HDAC5	10014	histone deacetylase 5	chromatin modeling; chromatin silencing; cytoplasm; histone deacetylase activity; nucleus; regulation of transcription, DNA-dependent
AA203710	0.005978656	XPO7	23039	exportin 7	
H69803	0.006018395				
N76529	0.006024				
N41764	0.006029704	DLG7	9787	discs, large homolog 7 (Drosophila)	biological_process unknown; cell-cell signaling; cellular_component unknown; molecular_function unknown

Appendix G. Genes significantly different after 15m MPP+ treatment +/- SN50.

GENBANK	PVALUE	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
T87888	4.54E-10	KIAA1046	22867	KIAA1046 protein	
R94499	9.77E-10	GNB5	10681	guanine nucleotide binding protein (G protein), beta 5	
R63498	4.09E-09	FLJ23563	79993	hypothetical protein FLJ23563	integral to membrane
H68885	9.58E-09	TSSC3	7262	tumor suppressing subtransferable candidate 3	apoptosis; imprinting
R19119	1.01E-08				
AA010141	1.23E-08	SERPINH1	871	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	heat shock response
H18190	2.29E-08	JAK1	3716	Janus kinase 1 (a protein tyrosine kinase)	ATP binding; cytoskeleton; intracellular signaling cascade; protein amino acid phosphorylation; protein-tyrosine kinase activity; transferase activity
H53827	2.39E-08				
R55491	3.73E-08				
H18298	4.04E-08				
W44529	4.92E-08	MMP2	4313	matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	calcium ion binding; collagen catabolism; extracellular matrix; extracellular space; gelatinase A activity; hydrolase activity; zinc ion binding
T79540	6.25E-08		253992	LOC253992	
H84257	7.13E-08				

H25578	7.76E-08				
H10796	8.25E-08	SMN1	6606	survival of motor neuron 1, telomeric	
T83013	8.72E-08	HGD	3081	homogentisate 1,2-dioxygenase (homogentisate oxidase)	
T93785	1.32E-07				
R15267	1.34E-07				
H63676	1.52E-07	OPA1	4976	optic atrophy 1 (autosomal dominant)	GTP binding; mitochondrion; motor activity; vision
R20145	1.56E-07				
R62213	1.57E-07				
R17538	2.15E-07	PABPC4	8761	poly(A) binding protein, cytoplasmic 4 (inducible form)	RNA binding; RNA catabolism; RNA processing; blood coagulation; cytoplasm; poly(A) binding; protein biosynthesis; response to pest/pathogen/parasite
H59552	2.28E-07				
H58631	2.48E-07				
R41363	2.58E-07				
T87535	2.68E-07				
R26954	3.09E-07	CTSD	1509	cathepsin D (lysosomal aspartyl protease)	cathepsin D activity; hydrolase activity; lysosome; pepsin A activity; proteolysis and peptidolysis
AA018742	3.66E-07	ISL1	3670	ISL1 transcription factor, LIM/homeodomain, (islet-1)	RNA polymerase II transcription factor activity; development; energy pathways; nucleus; regulation of transcription, DNA-dependent; transcription factor activity
H50657	3.78E-07				
H59765	4.77E-07	C14orf68	283600	chromosome 14 open reading frame 68	
H72049	4.84E-07				
H47518	4.96E-07				
H65775	5.19E-07				
R26325	5.74E-07				
R48610	6.16E-07	TTC7	57217	tetratricopeptide repeat domain 7	

H18495	7.34E-07				
H82521	9.90E-07	ATP6V0B	533	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit c''	ATP biosynthesis; hydrogen ion transporter activity; hydrogen-transporting two-sector ATPase activity; hydrolase activity; integral to membrane; proton transport; transporter activity
R60838	1.46E-06				
T79552	1.74E-06				
R20019	2.33E-06				
H45355	2.47E-06				
W68050	2.54E-06	LGALS1	3956	lectin, galactoside-binding, soluble, 1 (galectin 1)	apoptosis; heterophilic cell adhesion; sugar binding
R34114	2.81E-06				
H68441	3.08E-06	FLJ14054	79614	hypothetical protein FLJ14054	
H02590	3.20E-06				
R00907	3.23E-06	PLEKHG1	57480	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	
H29730	3.44E-06				
H71504	3.44E-06				
H68976	3.47E-06	BXDC1	84154	brix domain containing 1	nucleus
H48570	3.65E-06				
R54090	3.66E-06				
R20373	3.78E-06	TMP21	10972	transmembrane trafficking protein	ER to Golgi transport; Golgi apparatus; integral to plasma membrane; intracellular protein transport; membrane fraction; microsome; protein carrier activity; protein transporter activity
R19859	3.94E-06	MGC21874	93624	hypothetical protein MGC21874	
H59135	4.80E-06	SPP2	6694	secreted phosphoprotein 2, 24kDa	endopeptidase inhibitor activity; extracellular space; skeletal development
H73186	5.21E-06				
R51610	5.22E-06				
H48282	5.97E-06				
H60821	6.08E-06				
W37870	6.13E-06				

H09945	8.07E-06				
H19297	8.11E-06	EDIL3	10085	EGF-like repeats and discoidin I-like domains 3	calcium ion binding; cell adhesion; cell adhesion molecule activity; development; integrin binding
H86498	8.39E-06				
H23933	8.56E-06				
H48578	9.68E-06				
AA150238	9.78E-06				
R47938	9.78E-06	FLJ32096	148646	hypothetical protein FLJ32096	
H27352	9.93E-06	HRAS	3265	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	GTPase activity; cell motility; cell shape and cell size control; cell surface receptor linked signal transduction; chemotaxis; cytoplasm; histogenesis and organogenesis; peripheral plasma membrane protein; plasma membrane; regulation of cell cycle; signal transduction
R85191	9.96E-06	FLJ31364	146956	homolog of yeast EME1 endonuclease	
R22957	1.07E-05	pp9099	80301	PH domain-containing protein	
R77347	1.07E-05	PPIG	9360	peptidyl-prolyl isomerase G (cyclophilin G)	FK506-sensitive peptidyl-prolyl cis-trans isomerase; cyclophilin; cyclophilin-type peptidyl-prolyl cis-trans isomerase activity; isomerase activity; mRNA splicing; nucleoplasm; pre-mRNA splicing factor activity; protein folding
H03917	1.14E-05	INSR	3643	insulin receptor	ATP binding; carbohydrate metabolism; cell growth and/or maintenance; development; energy pathways; epidermal growth factor receptor activity; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; receptor signaling protein tyrosine kinase activity; signal transduction; transferase activity; transmembrane receptor protein tyrosine kinase signaling pathway; transmembrane receptor protein tyrosine kinase signaling protein activity
R07186	1.18E-05				

H00498	1.20E-05	PPP2R3A	5523	protein phosphatase 2 (formerly 2A), regulatory subunit B'', alpha	calcium ion binding; protein phosphatase type 2A, intrinsic regulator activity
R36086	1.38E-05				
R50698	1.41E-05	MGC23166	221504	hypothetical protein MGC23166	protein binding
R81839	1.52E-05	TXK	7294	TXK tyrosine kinase	ATP binding; cytoplasm; intracellular signaling cascade; non-membrane spanning protein tyrosine kinase activity; protein amino acid phosphorylation; transferase activity
H12681	1.53E-05	FCGR1A	2209	Fc fragment of IgG, high affinity Ia, receptor for (CD64)	immune response; integral to plasma membrane; phagocytosis, engulfment; receptor signaling protein activity; signal transduction
H99202	1.54E-05	MGC4126	84859	hypothetical protein MGC4126	
H84293	1.58E-05	SLC12A5	57468	solute carrier family 12, (potassium-chloride transporter) member 5	amino acid transport; amino acid-polyamine transporter activity; cell ion homeostasis; chloride transport; integral to membrane; ion transport; potassium ion transport; potassium:chloride symporter activity; sodium ion transport; symporter activity; transporter activity
H45241	1.62E-05	RPL41	6171	ribosomal protein L41	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); protein biosynthesis; structural constituent of ribosome
R68131	1.68E-05	SLC31A2	1318	solute carrier family 31 (copper transporters), member 2	copper ion transport; copper ion transporter activity; integral to plasma membrane; transport
N29429	1.77E-05	CGI-57	27013	hypothetical protein CGI-57	
R48603	1.78E-05	AGS3	26086	activator of G-protein signaling 3	

R41584	1.80E-05	KIAA0194	22993	KIAA0194 protein	DNA binding; nucleus; regulation of transcription, DNA-dependent
R26844	1.90E-05				
H09314	1.90E-05	PPP2CA	5515	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	CTD phosphatase activity; calcium-dependent protein serine/threonine phosphatase activity; hydrolase activity; magnesium-dependent protein serine/threonine phosphatase activity; manganese ion binding; myosin phosphatase activity; protein amino acid dephosphorylation; protein phosphatase type 2A complex; protein phosphatase type 2A, intrinsic catalyst activity; protein phosphatase type 2B activity; protein phosphatase type 2C activity; regulation of cell cycle
R60546	1.96E-05	CD63	967	CD63 antigen (melanoma 1 antigen)	integral to plasma membrane; lysosomal membrane
T65132	2.13E-05	SSTR1	6751	somatostatin receptor 1	G-protein signaling, coupled to cyclic nucleotide second messenger; cell-cell signaling; digestion; integral to plasma membrane; negative regulation of cell proliferation; response to nutrients; rhodopsin-like receptor activity; somatostatin receptor activity
R22946	2.20E-05				
H73170	2.24E-05				
H09088	2.44E-05				
H84096	2.45E-05				
H47146	2.57E-05	ERCC1	2067	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	DNA repair; embryogenesis and morphogenesis; endodeoxyribonuclease activity; nucleotide-excision repair; nucleus
R77382	2.68E-05	FLJ10276	55108	hypothetical protein FLJ10276	

H62447	2.69E-05	MASP1	5648	mannan-binding lectin serine protease 1 (C4/C2 activating component of Ra-reactive factor)	complement activation; serine-type endopeptidase activity
R87060	3.10E-05	GGCX	2677	gamma-glutamyl carboxylase	blood coagulation; gamma-glutamyl carboxylase activity; integral to membrane; ligase activity; membrane fraction; protein modification
N23779	3.13E-05	CD151	977	CD151 antigen	cell adhesion; integral to plasma membrane; membrane fraction
AA035641	3.36E-05	IGFBP2	3485	insulin-like growth factor binding protein 2, 36kDa	extracellular space; insulin-like growth factor binding; regulation of cell growth
H14586	3.46E-05	PRPS1	5631	phosphoribosyl pyrophosphate synthetase 1	kinase activity; lipoate-protein ligase B activity; magnesium ion binding; neurogenesis; nucleoside metabolism; nucleotide biosynthesis; purine base metabolism; ribonucleoside monophosphate biosynthesis; ribose-phosphate diphosphokinase activity; transferase activity
H02379	3.66E-05				
H57136	3.77E-05	FXYD1	5348	FXYD domain containing ion transport regulator 1 (phospholemman)	chloride channel activity; chloride transport; integral to plasma membrane; ion channel activity; ion transport; muscle contraction
T95699	3.89E-05	C11ORF4	56834	chromosome 11 hypothetical protein ORF4	
R76214	3.89E-05	PCDH16	8642	protocadherin 16 dachsous-like (Drosophila)	calcium ion binding; calcium-dependent cell-cell adhesion; cell adhesion; cell adhesion molecule activity; homophilic cell adhesion; integral to membrane
T86042	4.07E-05				

R14364	4.08E-05	PPM1E	22843	protein phosphatase 1E (PP2C domain containing)	
H26760	4.12E-05	KIAA0375	9853	KIAA0375 gene product	
H10327	4.21E-05	RAP1B	5908	RAP1B, member of RAS oncogene family	GTP binding; RAS small monomeric GTPase activity; membrane; small GTPase mediated signal transduction
AA133962	4.48E-05				
R73991	4.48E-05	KIAA1160	57461	KIAA1160 protein	
H27334	4.54E-05	DDR1	780	discoidin domain receptor family, member 1	ATP binding; cell adhesion; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; transferase activity; transmembrane receptor protein tyrosine kinase activity; transmembrane receptor protein tyrosine kinase signaling pathway
N/A1	4.56E-05				
H25699	4.78E-05	OIP2	11340	Opa-interacting protein 2	3'-5' exoribonuclease activity; RNA binding; biological_process unknown; cellular_component unknown; exonuclease activity; exosome (RNase complex); hydrolase activity; molecular_function unknown; nucleus; rRNA processing
H77390	4.89E-05	GOLGA1	2800	golgi autoantigen, golgin subfamily a, 1	
R19064	4.95E-05	LOC51275	51275	apoptosis-related protein PNAS-1	
R53020	4.95E-05				
T98139	5.04E-05	HLA-B	3106	major histocompatibility complex, class I, B	MHC class I receptor activity; antigen presentation, endogenous antigen; antigen processing, endogenous antigen via MHC class I; immune response; integral to plasma membrane
H62424	5.04E-05				
H58461	5.44E-05		339088	similar to My016 protein	

W72707	5.65E-05	PRDX6	9588	peroxiredoxin 6	antioxidant activity; cytosol; hydrolase activity; lipid catabolism; lysosome; non-selenium glutathione peroxidase activity; oxidoreductase activity; phospholipase A2 activity; phospholipid catabolism; response to oxidative stress
T50388	5.74E-05				
AA046291	5.79E-05				
R21970	5.86E-05	GTF2H2	2966	general transcription factor IIH, polypeptide 2, 44kDa	DNA repair; nucleus; regulation of transcription, DNA-dependent
H60460	6.01E-05	DCL-1	9936	type I transmembrane C-type lectin receptor DCL-1	heterophilic cell adhesion; sugar binding
N21532	6.47E-05				
R50087	6.67E-05	GREB1	9687	GREB1 protein	
T83091	7.10E-05				
H86672	7.16E-05				
H70974	7.39E-05				
H47346	7.80E-05	KMO	8564	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	aromatic compound metabolism; electron transport; electron transporter activity; kynurenine 3-monooxygenase activity
R40597	7.95E-05	WARS2	10352	tryptophanyl tRNA synthetase 2 (mitochondrial)	ATP binding; ligase activity; mitochondrion; soluble fraction; tryptophan-tRNA ligase activity; tryptophanyl-tRNA aminoacylation
H64609	8.04E-05	AHR	196	aryl hydrocarbon receptor	apoptosis; cell cycle; ligand-dependent nuclear receptor activity; nucleus; regulation of transcription, DNA-dependent; response to stress; response to xenobiotic stimulus; signal transduction; transcription factor activity; transcription from Pol II promoter
AA031465	8.06E-05	GEFT	115557	RAC/CDC42 exchange	

				factor	
T83168	8.41E-05				
R76162	8.68E-05	KRT23	25984	keratin 23 (histone deacetylase inducible)	
H84008	8.89E-05				
AA130221	9.40E-05	DSC3	1825	desmocollin 3	calcium ion binding; cell adhesion; cell adhesion molecule activity; cytoskeleton; homophilic cell adhesion; integral to membrane; intercellular junction; membrane fraction; plasma membrane
R18841	0.000101747	HNT	50863	neurotrimin	cell adhesion; cell adhesion molecule activity; integral to plasma membrane; neuronal cell recognition
AA142939	0.000102144	ATP8B2	57198	ATPase, Class I, type 8B, member 2	ATPase activity; cation transport; hydrolase activity; integral to membrane; magnesium ion binding; metabolism; phospholipid-translocating ATPase activity
R44307	0.000103006	PPP1R9B	84687	protein phosphatase 1, regulatory subunit 9B, spinophilin	intracellular signaling cascade; membrane; transport; transporter activity
T85558	0.000103649				
H83405	0.000104317	FGD1	2245	faciogenital dysplasia (Aarskog-Scott syndrome)	development; guanyl-nucleotide exchange factor activity; histogenesis and organogenesis; signal transduction; zinc ion binding
H40607	0.00010928				
AA031859	0.000109417	TIMM13	26517	translocase of inner mitochondrial membrane 13 homolog (yeast)	hearing; mitochondrial inner membrane pre-sequence translocase complex; mitochondrial translocation; mitochondrion; protein targeting; protein translocase activity; zinc ion binding
R08080	0.000110623				
H05011	0.00011312				
H84657	0.000118031	GRWD	83743	glutamate rich WD repeat	

				protein GRWD	
R71723	0.000120086	SLC4A2	6522	solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)	anion transport; anion transporter activity; antiporter activity; inorganic anion exchanger activity; integral to membrane; membrane fraction
H20790	0.000123136		348024	similar to TPIP alpha lipid phosphatase	
H13744	0.000125595	ALDOA	226	aldolase A, fructose-bisphosphate	
R00710	0.00012696				
H47539	0.000133883				
R15155	0.0001357				
H18199	0.000135845				
R52852	0.000136721				
R55009	0.000136973	GNAI2	2771	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	G-protein coupled receptor protein signaling pathway; GTP binding; heterotrimeric G-protein GTPase activity; negative regulation of adenylate cyclase activity; response to nutrients; signal transducer activity; signal transduction
R52023	0.000137073		340249	similar to hypothetical protein FLJ35882	
H54430	0.000142236				
H78668	0.000145452				

AA045053	0.000148 34	PTN	5764	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	cell proliferation; cytokine activity; extracellular space; growth factor activity; heparin binding; neurogenesis; positive regulation of cell proliferation; protein phosphatase inhibitor activity; regulation of cell cycle; transmembrane receptor protein tyrosine phosphatase signaling pathway
H61972	0.000149 717	PIN4	5303	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	FK506-sensitive peptidyl-prolyl cis-trans isomerase; cyclophilin; cyclophilin-type peptidyl-prolyl cis-trans isomerase activity; isomerase activity; mitochondrial matrix; protein folding
H59568	0.000154 717	WBP3	91010	WW domain binding protein 3	
H18504	0.000177 193	NEUROD 6	63974	neurogenic differentiation 6	DNA binding; nucleus; regulation of transcription, DNA-dependent
R72577	0.000179 49	FLJ11753	79712	hypothetical protein FLJ11753	
N76562	0.000181 883	FTH1	2495	ferritin, heavy polypeptide 1	binding; cell proliferation; ferric iron binding; ferritin complex; intracellular iron ion storage; iron ion transport
R76890	0.000185 627	KIAA1340	57542	KIAA1340 protein	
R91797	0.000186 22				
H52741	0.000188 793				
H11855	0.000191 519	ELK1	2002	ELK1, member of ETS oncogene family	
R89056	0.000191 859	LAMP1	3916	lysosomal-associated membrane protein 1	integral to plasma membrane; lysosome; membrane fraction
N55283	0.000192 731	KIAA0469	9903	KIAA0469 gene product	
W47525	0.000193 929				
R22402	0.000194 653				

H02088	0.000194866	RBAF600	23352	retinoblastoma-associated factor 600	
N34169	0.000199375	NF2	4771	neurofibromin 2 (bilateral acoustic neuroma)	cytoskeleton; hearing; negative regulation of cell cycle; negative regulation of cell proliferation; plasma membrane; structural molecule activity
R09418	0.000202042				
H82992	0.000210935	PIGT	51604	phosphatidyl inositol glycan class T	
H09429	0.000214377				
H60520	0.000218429				
R83017	0.000234786				
R88894	0.000237613				
AA152287	0.000250526	SLC35B2	347734	solute carrier family 35, member B2	copper ion binding; electron transport; electron transporter activity
H01640	0.000251997	PSG1	5669	pregnancy specific beta-1-glycoprotein 1	extracellular space; pregnancy
W72400	0.000253169	C12orf2	11228	chromosome 12 open reading frame 2	neuropeptide signaling pathway
N33037	0.000260259	ABT1	29777	activator of basal transcription 1	general RNA polymerase II transcription factor activity; nucleus; transcription co-activator activity; transcription from Pol II promoter
N95559	0.000262254	C20orf21	54915	chromosome 20 open reading frame 21	
R33026	0.000269822				
R61223	0.000269985	OACT1	154141	O-acyltransferase (membrane bound) domain containing 1	
R25152	0.000274				

	442				
H61842	0.000275864				
W95842	0.000278085	MARCKS	4082	myristoylated alanine-rich protein kinase C substrate	actin cross-linking activity; actin cytoskeleton; calmodulin binding; cell motility; plasma membrane
N49727	0.000280013	GDF11	10220	growth differentiation factor 11	cellular_component unknown; cytokine activity; growth factor activity; mesoderm development; neurogenesis; skeletal development
H78479	0.000293917	TXNL2	10539	thioredoxin-like 2	electron transport; electron transporter activity
H85095	0.000296065	POLR2J2	246721	DNA directed RNA polymerase II polypeptide J-related gene	DNA binding; DNA-directed RNA polymerase activity; transcription
H16042	0.000297025				
R84724	0.000300636	ABCA3	21	ATP-binding cassette, sub-family A (ABC1), member 3	ATP binding; ATP-binding cassette (ABC) transporter activity; drug resistance; integral to membrane; membrane fraction; nucleotide binding; transport; transporter activity
N33492	0.000304628	STHM	10610	sialyltransferase	Golgi apparatus; integral to membrane; protein amino acid glycosylation; sialyltransferase activity; transferase activity, transferring glycosyl groups
H18220	0.000310781				
W55993	0.000310 94	FBN2	2201	fibrillin 2 (congenital contractural arachnodactyly)	calcium ion binding; embryogenesis and morphogenesis; extracellular matrix; extracellular matrix structural constituent; histogenesis and organogenesis
R14286	0.000312815				
R27036	0.000316911				
H49310	0.000319938	WBSCR24	155382	Williams Beuren syndrome chromosome region 24	
T65549	0.000323563	PPP1R2	5504	protein phosphatase 1, regulatory	energy pathways; glycogen metabolism; type 1 serine/threonine specific protein phosphatase inhibitor activity

				(inhibitor) subunit 2	
N89592	0.000325074	TUBB2	10383	tubulin, beta, 2	GTP binding; MHC class I protein binding; chaperone activity; cytoskeleton; microtubule-based movement; natural killer cell mediated cytolysis; structural constituent of cytoskeleton; tubulin
R09905	0.000325399				
AA099381	0.000330749	COX15	1355	COX15 homolog, cytochrome c oxidase assembly protein (yeast)	cytochrome-c oxidase activity; electron transporter activity; mitochondrion; respiratory gaseous exchange
H54691	0.00033705	ARHGEF12	23365	Rho guanine nucleotide exchange factor (GEF) 12	intracellular signaling cascade; signal transducer activity
N92911	0.000338021	DJ473B4	56180	hypothetical protein dJ473B4	structural molecule activity
R51898	0.00034218				
H50471	0.000345409	PDCD6	10016	programmed cell death 6	apoptosis; calcium ion binding; induction of apoptosis by extracellular signals
R50932	0.000346131	D4ST-1	113189	dermatan-4-sulfotransferase-1	transferase activity
H62898	0.00034619				
H49225	0.00035608				
H83857	0.000362813				
H52939	0.000374234				
R54918	0.000382138	FLJ13912	64785	hypothetical protein FLJ13912	
R21825	0.00038778				
H59238	0.000395119	RARRES2	5919	retinoic acid receptor responder	cellular_component unknown; molecular_function unknown; retinoid metabolism

				(tazarotene induced) 2	
H50385	0.000397598	CSEN	30818	calsenilin, presenilin binding protein, EF hand transcription factor	DNA binding; calcium ion binding; regulation of transcription from Pol II promoter; signal transduction; transcription co-repressor activity
R28090	0.000402226	KIAA1495	57631	KIAA1495 protein	
AA142924	0.000405923	DF	1675	D component of complement (adipsin)	chymotrypsin activity; complement activation, alternative pathway; complement factor D activity; hydrolase activity; proteolysis and peptidolysis; trypsin activity
AA098963	0.000410221	SLC1A5	6510	solute carrier family 1 (neutral amino acid transporter), member 5	dicarboxylic acid transport; integral to plasma membrane; membrane fraction; neutral amino acid transport; neutral amino acid transporter activity; receptor activity; sodium:dicarboxylate/tricarboxylate symporter activity; transport
N45640	0.000411849	CH25H	9023	cholesterol 25-hydroxylase	catalytic activity; lipid metabolism; membrane fraction; steroid hydroxylase activity
R79518	0.000419 88	MCAM	4162	melanoma cell adhesion molecule	cell adhesion; cell adhesion molecule activity; embryogenesis and morphogenesis; integral to plasma membrane
H65175	0.000426372	SLC31A1	1317	solute carrier family 31 (copper transporters), member 1	copper ion transport; copper ion transporter activity; integral to plasma membrane; transport
H27034	0.000427779	IGKC	3514	immunoglobulin kappa constant	antigen binding; immune response
R69282	0.000445804	RSN	6249	restin (Reed-Steinberg cell-expressed intermediate filament-associated	endosome; intermediate filament; kinesin complex; microtubule binding; microtubule cytoskeleton; microtubule-based process; nonselective vesicle transport; nucleic acid binding

				protein)	
T96973	0.000446949				
H00760	0.000450476				
H84224	0.000451897				
R23351	0.00045636				
R88435	0.000463204	DPP6	1804	dipeptidylpeptidase 6	catalytic activity; dipeptidyl-peptidase IV activity; dipeptidyl-peptidase activity; integral to membrane; proteolysis and peptidolysis
H45746	0.000474872				
N64478	0.00048103	HUMYZ82H07	29792	hypothetical protein HUMYZ82H07	
R60030	0.000485024	KIAA0972	22869	KIAA0972 protein	DNA binding; nucleus; regulation of transcription, DNA-dependent
W80519	0.000485204	SDBCAG84	51614	serologically defined breast cancer antigen 84	
R08165	0.000487527				
R85044	0.00049781	SMPD1	6609	sphingomyelin phosphodiesterase 1, acid lysosomal (acid sphingomyelinase)	carbohydrate metabolism; hydrolase activity, acting on glycosyl bonds; lysosome; neurogenesis; signal transduction; sphingomyelin metabolism; sphingomyelin phosphodiesterase activity
H16242	0.000498382	SDCCAG16	10813	serologically defined colon cancer antigen 16	tumor antigen
H59136	0.000506347	CYP39A1	51302	cytochrome P450, family 39, subfamily A, polypeptide 1	bile acid biosynthesis; bile acid catabolism; digestion; electron transport; electron transporter activity; endoplasmic reticulum; membrane; microsome; monooxygenase activity; oxysterol 7-alpha-hydroxylase activity
R10571	0.000528464				

H27559	0.000539128	C16orf35	8131	chromosome 16 open reading frame 35	biological_process unknown; cellular_component unknown; molecular_function unknown
N62241	0.000545189	FLJ32029	283209	hypothetical protein FLJ32029	carbohydrate metabolism; intramolecular transferase activity, phosphotransferases
H14999	0.000547702	ARHGEF6	9459	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	GTPase activator activity; JNK cascade; Rho guanyl-nucleotide exchange factor activity; Rho interactor activity; apoptosis; intracellular
H58957	0.000561922	MCL1	4170	myeloid cell leukemia sequence 1 (BCL2-related)	apoptotic program; development; heat shock response
T81715	0.000562093				
N95586	0.000564814	TPD52	7163	tumor protein D52	embryogenesis and morphogenesis; kinesin complex
R06552	0.000566932				
T92003	0.000589798	KIAA0342	9881	KIAA0342 gene product	DNA binding; membrane; nucleus; transport; transporter activity
H10658	0.000592146				
N/A1	0.000611815				
H63763	0.000624428				
AA059213	0.000647758				
W32895	0.000661226	JUN	3725	v-jun sarcoma virus 17 oncogene homolog (avian)	RNA polymerase II transcription factor activity; cell growth and/or maintenance; nuclear chromosome; regulation of transcription, DNA-dependent; transcription factor activity
H47026	0.000674021	MGAT3	4248	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	Golgi apparatus; N-linked glycosylation; beta-1,4-mannosylglycoprotein 4-beta-N-acetylglucosaminyltransferase activity; integral to membrane; transferase activity, transferring glycosyl groups
T65291	0.000691	DKFZp76	222865	hypothetic	

	869	1L1417		al protein DKFZp761L1417	
N68416	0.000695289				
N80976	0.000700273	LOC51252	51252	hypothetical protein LOC51252	
R19153	0.000705028	RPS7	6201	ribosomal protein S7	RNA binding; cytosolic small ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; ribosome; structural constituent of ribosome
R99067	0.000710473				
R85150	0.000724255	EPHB6	2051	EphB6	ATP binding; ephrin receptor activity; integral to membrane; protein amino acid phosphorylation; protein-tyrosine kinase activity; receptor activity; transmembrane receptor protein tyrosine kinase signaling pathway
H79433	0.000728587				
H71072	0.000730464				
R87193	0.000760245				
H18883	0.000763027				
H50015	0.00079172				
R87345	0.000803526	MGC2656	79414	hypothetical protein MGC2656	
R88587	0.000839108				
W87783	0.000839432	REV1L	51455	REV1-like (yeast)	DNA repair; intracellular; transferase activity
H14566	0.00084572				
H46055	0.000864372	KIAA0725	23259	KIAA0725 protein	metal ion binding
H43816	0.00086502				
N72553	0.000867797	CALM3	808	calmodulin 3 (phosphorylase kinase, delta)	calcium ion binding

H44725	0.000868955	PAWR	5074	PRKC, apoptosis, WT1, regulator	apoptosis; cytoplasm; negative regulation of cell proliferation; negative regulation of transcription from Pol II promoter; nucleus; transcription co-repressor activity
H17327	0.000891169	PM5	23420	pM5 protein	
AA059242	0.000899662				
H24891	0.000908478				
R07137	0.000911983	HIC2	23119	hypermethylated in cancer 2	DNA binding; negative regulation of transcription, DNA-dependent; nucleus; protein C-terminus binding
T86313	0.000914196	MAOB	4129	monoamine oxidase B	amine oxidase (flavin-containing) activity; electron transport; electron transporter activity; integral to membrane; mitochondrial membrane; oxidoreductase activity
AA054137	0.000916815				
H43746	0.000931318	TRIP8	221037	thyroid hormone receptor interactor 8	intracellular; ligand-dependent thyroid hormone receptor interactor activity; regulation of transcription, DNA-dependent
H41330	0.000937356	LRRC2	79442	leucine-rich repeat-containing 2	
H90964	0.000973406	STRN4	29888	striatin, calmodulin binding protein 4	calmodulin binding; cytoplasm; kinesin complex; membrane fraction; signal transduction; structural molecule activity; synaptic transmission
R24476	0.000995792				
H28503	0.000996126				
H26552	0.001011054	MGC5395	79026	hypothetical protein MGC5395	intracellular signaling cascade
H42894	0.001028578	KIAA0420	9717	KIAA0420 gene product	intracellular; transport; transporter activity
R23778	0.001030233	C7	730	complement component 7	complement activation, alternative pathway; complement activation, classical pathway; complement activity; cytolysis; immune response; integral to membrane; membrane attack complex; response to pathogenic bacteria
W80487	0.001053456	DC50	81892	hypothetical protein	nucleic acid binding

				DC50	
H61030	0.001061303				
H29610	0.001065284				
R27269	0.001066377				
H43455	0.001071695	PP2447	80305	hypothetical protein PP2447	
T74007	0.0010728	NCSTN	23385	nicastrin	integral to membrane; molecular_function unknown; proteolysis and peptidolysis
H38593	0.001081703				
R92948	0.001093574				
AA149222	0.001115667	MGC14836	92014	hypothetical protein similar to CG7943	binding; mitochondrial inner membrane; transport
N91376	0.001119322	KIAA0247	9766	KIAA0247 gene product	integral to membrane
R13021	0.001136897	FLJ10751	55222	hypothetical protein FLJ10751	
T80602	0.00113961				
R59528	0.001213546				
AA045734	0.001227907	BET1L	51272	blocked early in transport 1 homolog (S. cerevisiae) like	
T97903	0.001241936				
H93450	0.001256746	ZNF347	84671	zinc finger protein 347	DNA binding; nucleus; regulation of transcription, DNA-dependent
AA136161	0.001288343				
N33229	0.001288878				
AA026902	0.001289046	FLJ11320	55343	GDP-fucose transporter 1	Golgi apparatus; integral to membrane; sugar porter activity; transport
T84539	0.001311256				
R78049	0.001327308	SUMF2	25870	sulfatase modifying factor 2	
N73749	0.001342171				

N21091	0.001342 53	CAV1	857	caveolin 1, caveolae protein, 22kDa	caveola; integral to plasma membrane; structural molecule activity
N29422	0.001354 1	MMP2	4313	matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	calcium ion binding; collagen catabolism; extracellular matrix; extracellular space; gelatinase A activity; hydrolase activity; zinc ion binding
N94626	0.001371 637	SNRPD2	6633	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	pre-mRNA splicing factor activity; small nuclear ribonucleoprotein complex; small nucleolar ribonucleoprotein complex; spliceosome assembly; spliceosome complex
AA126875	0.001387 999	FYCO1	79443	FYVE and coiled-coil domain containing 1	zinc ion binding
R99223	0.001391 131				
H04901	0.001422 235	MKNK1	8569	MAP kinase-interacting serine/threonine kinase 1	ATP binding; cAMP-dependent protein kinase activity; protein amino acid phosphorylation; protein kinase CK2 activity; protein kinase cascade; protein serine/threonine kinase activity; regulation of protein biosynthesis; regulation of translation; response to stress; transferase activity
R64592	0.001428 63				
H71213	0.001430 042	F2	2147	coagulation factor II (thrombin)	STAT protein nuclear translocation; acute-phase response; apoptosis; blood coagulation; calcium ion binding; caspase activation; chymotrypsin activity; development; extracellular space; hydrolase activity; proteolysis and peptidolysis; regulation of cell cycle; response to wounding; soluble fraction; thrombin activity; trypsin activity; tyrosine phosphorylation of STAT protein
R98591	0.001441 915				
R11336	0.001449 014				

H11235	0.001468172	PEX11A	8800	peroxisomal biogenesis factor 11A	integral to peroxisomal membrane; peroxisome organization and biogenesis; signal transduction
R83560	0.001491671				
R14363	0.00150008	HDAC5	10014	histone deacetylase 5	chromatin modeling; chromatin silencing; cytoplasm; histone deacetylase activity; nucleus; regulation of transcription, DNA-dependent
W79028	0.001511113	RPE	6120	ribulose-5-phosphate-3-epimerase	ribulose-phosphate 3-epimerase activity
H65832	0.001524908				
H93200	0.001527254				
T96118	0.001545223				
W24831	0.00155314	DPT	1805	dermatopontin	cell adhesion; cell adhesion molecule activity; extracellular matrix; protein binding
R76832	0.001558703	ATP5J2	9551	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2	ATP biosynthesis; hydrogen ion transporter activity; hydrogen-transporting two-sector ATPase activity; mitochondrion; proton transport
AA193482	0.001568499	FLJ12287	64218	hypothetical protein FLJ12287 similar to semaphorins	development; integral to membrane; neurogenesis; receptor activity
T53075	0.001585488	ADCY5	111	adenylate cyclase 5	cAMP biosynthesis; calcium/calmodulin-responsive adenylate cyclase activity; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; magnesium ion binding
H70392	0.001614073	DDX52	11056	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52	
R87198	0.00162329	TUBB5	10382	tubulin, beta, 5	cytoskeleton; structural constituent of cytoskeleton
AA046082	0.001623832				

N70099	0.001626892	OSBP2	23762	oxysterol binding protein 2	lipid transport; membrane; steroid metabolism
R88980	0.001651243	LOC348262	348262	hypothetical protein LOC348262	
N90792	0.001664459				
R98461	0.001667167	SMC5L1	23137	SMC5 structural maintenance of chromosomes 5-like 1 (yeast)	ATP binding; chromosome segregation; nucleus
H48488	0.001669186				
R36523	0.001690459	NRP2	8828	neuropilin 2	axon guidance; membrane fraction; receptor activity; vascular endothelial growth factor receptor activity
W48584	0.001721581	P4HA2	8974	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	electron transporter activity; endoplasmic reticulum; oxidoreductase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors; oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; procollagen-proline 4-dioxygenase activity; protein metabolism
W76572	0.001725886	NUDT1	4521	nudix (nucleoside diphosphate linked moiety X)-type motif 1	8-oxo-7,8-dihydroguanine triphosphatase activity; DNA repair; GTPase activity; hydrolase activity; response to oxidative stress
T79650	0.001781352	ARVCF	421	armadillo repeat gene deletes in velocardiofacial syndrome	cell adhesion; cell adhesion molecule activity; cytoskeleton; development; intracellular; kinesin complex; structural molecule activity
AA114945	0.001792828				
T99576	0.001796				

	991				
T96928	0.00179953				
T89571	0.001813156				
N30288	0.001814543	PFDN2	5202	prefoldin 2	chaperone activity; prefoldin complex; protein folding
AA128562	0.001816196				
R73050	0.001833621	CNTFR	1271	ciliary neurotrophic factor receptor	GPI-anchored membrane-bound receptor; ciliary neurotrophic factor receptor activity; membrane; neurogenesis; receptor activity; signal transduction
H69787	0.001837022				
N25523	0.001869222	HSPE1	3336	heat shock 10kDa protein 1 (chaperonin 10)	co-chaperonin activity; heat shock protein activity; mitochondrion; protein folding
R86231	0.001881771	PC326	55827	PC326 protein	
N68871	0.001908816				
R49189	0.001912823	SLC30A6	55676	solute carrier family 30 (zinc transporter), member 6	
H26200	0.001942936		349268	similar to hypothetical protein LOC286286	
R26108	0.00195087				
R14326	0.002010252	HERC1	8925	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1	ARF guanyl-nucleotide exchange factor activity; Golgi apparatus; catalytic activity; nonselective vesicle transport; ubiquitin cycle; ubiquitin-protein ligase activity
AA034144	0.002010937	CD36	948	CD36 antigen (collagen type I receptor, thrombosp	blood coagulation; cell adhesion; cell adhesion molecule activity; fatty acid metabolism; integral to plasma membrane; membrane fraction; receptor activity; transport

				ondin receptor)	
R88895	0.002028496	MANBAL	63905	mannosidase, beta A, lysosomal-like	integral to membrane
H50436	0.002063585	ALDH6A1	4329	aldehyde dehydrogenase 6 family, member A1	metabolism; methylmalonate-semialdehyde dehydrogenase (acylating) activity; mitochondrion; oxidoreductase activity; pyrimidine nucleotide metabolism; valine metabolism
T48772	0.002074625	RPL12	6136	ribosomal protein L12	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); intracellular; protein biosynthesis; ribosome; structural constituent of ribosome
AA204653	0.002107156				
H59454	0.002111161				
T64848	0.002141857	PER3	8863	period homolog 3 (Drosophila)	nucleus; regulation of transcription, DNA-dependent; rhythmic behavior; signal transducer activity; signal transduction
H65366	0.002145291				
H80680	0.002172772				
R48615	0.002214369	C14orf21	161424	chromosome 14 open reading frame 21	RNA binding
H28534	0.002243158	AQP1	358	aquaporin 1 (channel-forming integral protein, 28kDa)	excretion; integral to plasma membrane; transport; water transport; water transporter activity
H83025	0.002258751				
R43017	0.002265502				
W47347	0.00230225	ABCF3	55324	ATP-binding cassette, sub-family F (GCN20), member 3	

N34930	0.002336485	DLL3	10683	delta-like 3 (Drosophila)	N signaling pathway; Notch binding; calcium ion binding; cell differentiation; cell fate determination; embryonic development (sensu Mammalia); integral to membrane; neurogenesis; skeletal development
H14143	0.002349457	ATP1A3	478	ATPase, Na+/K+ transporting, alpha 3 polypeptide	ATP binding; hydrolase activity; integral to membrane; magnesium ion binding; metabolism; monovalent inorganic cation transporter activity; potassium ion transport; sodium ion transport; sodium/potassium-exchanging ATPase activity; sodium/potassium-exchanging ATPase complex; transport
AA028961	0.002366334				
R66188	0.002371586				
H21568	0.002379889	CNNM3	26505	cyclin M3	
N64492	0.002388716				
H63711	0.002410236				
R09962	0.002421401	PMS2L6	5384	postmeiotic segregation increased 2-like 6	damaged DNA binding; mismatch repair; nucleus
R43540	0.002429377				
AA004343	0.002454559				
W32324	0.002505773	GPX1	2876	glutathione peroxidase 1	glutathione peroxidase activity; oxidoreductase activity; response to oxidative stress
H38541	0.002515044	CNOT3	4849	CCR4-NOT transcription complex, subunit 3	
W96066	0.002515087	ACTC	70	actin, alpha, cardiac muscle	actin filament; motor activity; muscle contraction; muscle development; regulation of heart rate; structural constituent of cytoskeleton; structural constituent of muscle
N/A2	0.002532621				
R31317	0.0025551				
T79362	0.002635				

	534				
T87194	0.002640554				
H46579	0.002641187	LOC284611	284611	hypothetical protein LOC284611	
W57989	0.002661555	TRIM2	23321	tripartite motif-containing 2	biological_process unknown; cytoplasm; myosin binding; zinc ion binding
H46133	0.002673406	BAI2	576	brain-specific angiogenesis inhibitor 2	G-protein coupled receptor activity; integral to membrane; neuropeptide signaling pathway
H43625	0.002710629	LOC220074	220074	Hypothetical 55.1 kDa protein F09G8.5 in chromosome III	
N63546	0.00271226				
H39920	0.002718032				
H89087	0.002721451	RNPS1	10921	RNA binding protein S1, serine-rich domain	RNA binding; RNA splicing; nucleus; transcription
N39391	0.002748964	MGC14799	84296	hypothetical protein MGC14799	
W79479	0.002751486	AP2M1	1173	adaptor-related protein complex 2, mu 1 subunit	clathrin vesicle coat; coated pit; intracellular protein transport; nonselective vesicle transport; secretory vesicle; transporter activity
H47114	0.002765108				
N74558	0.002774032				
N62283	0.002775021	TRAM1	23471	translocation associated membrane protein 1	cotranslational membrane targeting; endoplasmic reticulum; endoplasmic reticulum receptor activity; integral to membrane; protein targeting
R45627	0.002782889				
R18705	0.002796542	DKFZp761C169	65056	vasculin	

R47859	0.002825359	NPR1	4881	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	ATP binding; cGMP biosynthesis; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; peptide receptor activity, G-protein coupled; protein amino acid phosphorylation; protein kinase activity; receptor activity; receptor guanylate cyclase activity; regulation of blood pressure
AA125889	0.002828 64	PTMS	5763	parathymosin	DNA replication; cellular defense response; development; nucleus; regulation of cell cycle
N64488	0.002852346	PHTF1	10745	putative homeodomain transcription factor 1	nucleus; regulation of transcription, DNA-dependent; transcription factor activity
T91335	0.002881994	LUC7L	55692	LUC7-like (S. cerevisiae)	
R83014	0.002892845				
H84604	0.002928862	SLC21A12	28231	solute carrier family 21 (organic anion transporter), member 12	integral to membrane; ion transport; transporter activity
AA115064	0.002933016				
H59405	0.002940249	FLJ10298	54682	hypothetical protein FLJ10298	
H61036	0.002943 42				
AA034109	0.003019 47	MINK	50488	misshapen/NIK-related kinase	ATP binding; cAMP-dependent protein kinase activity; development; protein amino acid phosphorylation; protein kinase CK2 activity; protein kinase cascade; protein serine/threonine kinase activity; response to stress; small GTPase regulatory/interacting protein activity; transferase activity
N74414	0.003068 48	SDCCAG3	10807	serologically defined colon cancer antigen 3	tumor antigen
W32375	0.003103				

	289				
T87509	0.003115178				
N42162	0.003128355	DC2	58505	DC2 protein	
N72164	0.003137146				
H10026	0.00314539	ZNF193	7746	zinc finger protein 193	nucleus; protein binding; regulation of transcription, DNA-dependent; transcription factor activity
T97703	0.00315648				
AA031950	0.003182394				
T92612	0.003193663	CEPT1	10390	choline/ethanolamine phosphotransferase	ethanolaminephosphotransferase activity; integral to membrane; lipid metabolism; phospholipid biosynthesis; transferase activity
T52361	0.003197303	DKFZP434P1750	26000	DKFZP434P1750 protein	catalytic activity; metabolism
T70299	0.003226285				
T86284	0.003273129				
H45174	0.003288345	CLMN	79789	calmin (calponin-like, transmembrane)	
R19599	0.003351854				
R39421	0.003373338	PIGM	93183	phosphatidylinositol glycan, class M	transferase activity
R89424	0.00338303				
AA028907	0.003403016		153603	LOC153603	
R07810	0.003407136				
T84788	0.003432787				
R82834	0.00345738				
H56035	0.003463096	FLJ31842	148534	hypothetical protein FLJ31842	
H20520	0.003478042				
AA210905	0.003491				

	026				
R94248	0.003493034				
H62020	0.003494706				
N93517	0.003504284				
T77987	0.003518125		345466	similar to 6-pyruvoyl-tetrahydropterin synthase	
N42817	0.003546932	COX6C	1345	cytochrome c oxidase subunit VIc	aa3-type cytochrome c oxidase; ba3-type cytochrome c oxidase; caa3-type cytochrome c oxidase; cbb3-type cytochrome c oxidase; cytochrome-c oxidase activity; electron transport; energy pathways; inner membrane; mitochondrion; oxidoreductase activity
AA047157	0.003561795	KAI1	3732	kangai 1 (suppression of tumorigenicity 6, prostate; CD82 antigen (R2 leukocyte antigen, antigen detected by monoclonal and antibody IA4))	integral to plasma membrane
AA125808	0.003584725	CAPS	828	calcyphosine	calcium ion binding; intracellular signaling cascade
H40811	0.003658811				
AA045369	0.003691143				
H22064	0.003703929	PHF12	57649	PHD finger protein 12	DNA binding; metabolism; oxidoreductase activity; regulation of transcription, DNA-dependent
R17223	0.003712105				
H77938	0.003713497	ETF1	2107	eukaryotic translation termination factor 1	RNA binding; cytoplasm; regulation of translational termination; translation release factor activity, codon specific
H69440	0.003723647	ANKRD13	88455	ankyrin repeat domain 13	

H78795	0.003736927	HAND2	9464	heart and neural crest derivatives expressed 2	angiogenesis; development; heart development; nucleus; regulation of transcription, DNA-dependent; transcription factor activity; transcription from Pol II promoter
N63947	0.003746113	FLJ21940	64848	hypothetical protein FLJ21940	
H08918	0.003746482	LMLN	89782	leishmanolysin-like (metallopeptidase M8 family)	
R48041	0.003759537	GAA	2548	glucosidase, alpha; acid (Pompe disease, glycogen storage disease type II)	alpha-glucosidase activity; carbohydrate metabolism; energy pathways; glycogen catabolism; hydrolase activity, hydrolyzing O-glycosyl compounds; lysosome
AA121519	0.003763832	PCSK7	9159	proprotein convertase subtilisin/kexin type 7	integral to Golgi membrane; peptidase activity; peptide hormone processing; proteolysis and peptidolysis; subtilase activity
R30807	0.003804148				
R97635	0.003807487				
W33113	0.00386657				
H69011	0.003868629	SKIL	6498	SKI-like	cell differentiation; cell growth and/or maintenance; molecular_function unknown; nucleus
T79962	0.003893833				
H45972	0.003905578				
R90824	0.003908283	TMEM10	93377	transmembrane protein 10	integral to membrane
H99439	0.003915936				
AA210785	0.003917258				

W47101	0.003926794	IL1B	3553	interleukin 1, beta	antimicrobial humoral response (sensu Invertebrata); apoptosis; cell proliferation; cell-cell signaling; extracellular space; immune response; inflammatory response; interleukin-1 receptor antagonist activity; negative regulation of cell proliferation; regulation of cell cycle; signal transducer activity; signal transduction
H53118	0.003979598				
R70072	0.003987828	ELN	2006	elastin (supravalvular aortic stenosis, Williams-Beuren syndrome)	cell proliferation; cell shape and cell size control; circulation; extracellular matrix; extracellular matrix structural constituent; extracellular space; histogenesis and organogenesis; respiratory gaseous exchange
R24502	0.004002844	ADSSL1	122622	adenylosuccinate synthase like 1	GTP binding; adenylosuccinate synthase activity; ligase activity; purine nucleotide biosynthesis
H69845	0.004013994				
H67054	0.004041873	OLR1	4973	oxidised low density lipoprotein (lectin-like) receptor 1	circulation; heterophilic cell adhesion; integral to plasma membrane; membrane fraction; proteolysis and peptidolysis; receptor activity; sugar binding
W15268	0.004087506	ARHA	387	ras homolog gene family, member A	GTP binding; Rho protein signal transduction; Rho small monomeric GTPase activity; actin cytoskeleton organization and biogenesis; cell growth and/or maintenance; cytoskeleton
AA025089	0.004101469	VDAC1	7416	voltage-dependent anion channel 1	anion transport; apoptogenic cytochrome c release channel activity; apoptotic program; integral to membrane; mitochondrial outer membrane; mitochondrion; voltage-dependent anion channel porin activity; voltage-dependent ion-selective channel activity
R68198	0.004130001				
H65231	0.004212748				
H51160	0.004249526	PPP2R1A	5518	protein phosphatase 2 (formerly 2A),	protein phosphatase type 2A activity

				regulatory subunit A (PR 65), alpha isoform	
AA037284	0.004277598	APRT	353	adenine phosphoribosyltransferase	adenine phosphoribosyltransferase activity; adenine salvage pathway; nucleoside metabolism; transferase activity, transferring glycosyl groups
H73751	0.004281436	MAP3K6	9064	mitogen-activated protein kinase kinase kinase 6	MAP kinase kinase kinase activity; activation of JUNK; signal transduction
H85811	0.00429958	HIPK2	28996	homeodomain interacting protein kinase 2	nucleus; protein kinase activity; transcription co-repressor activity
H04530	0.004311455	ECHS1	1892	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	energy pathways; fatty acid beta-oxidation; fatty acid metabolism; long-chain-enoyl-CoA hydratase activity; lyase activity; mitochondrion
N33550	0.00432996				
H27097	0.004331377	LOC338645	338645	hypothetical protein LOC338645	
H62770	0.004355849				
AA099281	0.00436511	COL18A1	80781	collagen, type XVIII, alpha 1	cell adhesion; cell adhesion molecule activity; collagen; extracellular matrix structural constituent; histogenesis and organogenesis; negative regulation of cell proliferation; vision
AA134572	0.004368155				
AA034076	0.004373584				
R63205	0.004380177				
R83247	0.004394632	GLB1	2720	galactosidase, beta 1	beta-galactosidase activity; lysosome
H08266	0.004422345	H2AV	94239	histone H2A.F/Z variant	

T86338	0.004449624				
R88711	0.004475335				
AA036800	0.004489289	IHPK3	117283	inositol hexaphosphate kinase 3	inositol-trisphosphate 3-kinase activity
H83488	0.00449527				
H06830	0.004560081				
AA053136	0.004589225	KIAA1982	170960	KIAA1982 protein	

Appendix H. Genes significantly different after 90m MPP+ treatment +/- PTIO.

GENBANK	PVALUE	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
R64526	4.25E-08				
R39393	4.84E-08				
H14810	2.84E-07		158819	hypothetical gene supported by AK057191; AL117536	
R11718	3.29E-07	TCF4	6925	transcription factor 4	DNA binding; RNA polymerase II transcription factor activity; nucleus; regulation of transcription from Pol II promoter
W58007	5.31E-07		339299	LOC339299	
H03447	6.23E-07				
R98825	6.82E-07	LOC283596	283596	hypothetical protein LOC283596	
H84229	3.46E-06				
T83371	4.28E-06				
N72164	7.33E-06				
AA029936	8.50E-06	PRKAR1B	5575	protein kinase, cAMP-dependent, regulatory, type I, beta	3',5'-cAMP binding; cAMP-dependent protein kinase complex; cAMP-dependent protein kinase, intrinsic regulator activity; protein amino acid phosphorylation; signal transduction
H84599	1.04E-05				
H12977	1.17E-05	PRKCG	5582	protein kinase C, gamma	ATP binding; cAMP-dependent protein kinase activity; calcium ion binding; diacylglycerol binding; intracellular signaling cascade; protein amino acid phosphorylation; protein kinase C activity; protein kinase CK2 activity; protein-tyrosine kinase activity; transferase activity
R56037	1.20E-05				
R28465	1.68E-05		221922	hypothetical gene supported by AL713633; BC014395	
T83702	1.71E-05				
T78466	1.73E-05	PSG5	5673	pregnancy specific beta-1-glycopr	extracellular space; plasma glycoprotein; pregnancy

				otein 5	
AA026351	1.94E-05				
T84214	1.97E-05				
H12575	2.18E-05	MGC8902	284565	hypothetical protein MGC8902	
R43469	2.68E-05	EPHB3	2049	EphB3	ATP binding; ephrin receptor activity; integral to plasma membrane; protein amino acid phosphorylation; receptor activity; signal transduction; transferase activity; transmembrane receptor protein tyrosine kinase signaling pathway
H20004	2.75E-05	WDR8	49856	WD repeat domain 8	
AA129918	2.95E-05	FLJ10385	55135	hypothetical protein FLJ10385	
AA046498	3.36E-05		347868	similar to hypothetical protein BC015353	
T87122	3.53E-05				
R26558	4.44E-05	SDCCAG10	10283	serologically defined colon cancer antigen 10	
W01227	4.64E-05	HDGF	3068	hepatoma-derived growth factor (high-mobility group protein 1-like)	cell proliferation; cytoplasm; extracellular space; growth factor activity; heparin binding; signal transduction
R05508	4.97E-05	HSPC163	29097	HSPC163 protein	
AA039224	5.09E-05				
R51914	5.34E-05	CGI-87	51112	CGI-87 protein	
T66875	6.08E-05				
R42763	6.49E-05	KIAA0319	9856	KIAA03	

				19 gene product	
W32438	7.02E-05	CRABP2	1382	cellular retinoic acid binding protein 2	epidermal differentiation; lipid binding; regulation of transcription, DNA-dependent; retinoid binding; signal transduction; transport; transporter activity
R06569	7.28E-05				
H62185	7.96E-05	LOC56965	56965	hypothetical protein from EUROIMAGE 1977056	
T84202	8.84E-05	TAPBP	6892	TAP binding protein (tapasin)	MHC-interacting protein; endoplasmic reticulum; endoplasmic reticulum membrane; immune response; integral to membrane; peptide antigen transporter activity; protein binding; protein complex assembly
R21465	9.03E-05	MAPT	4137	microtubule-associated protein tau	apoptosis; cytosol; microtubule associated complex; microtubule cytoskeleton organization and biogenesis; plasma membrane; structural constituent of cytoskeleton
H70162	0.00010865				
N64388	0.00010927	NR4A1	3164	nuclear receptor subfamily 4, group A, member 1	DNA binding; ligand-dependent nuclear receptor activity; signal transduction
H85859	0.000122345	CPR8	9236	cell cycle progression 8 protein	
H15158	0.000127648	HSPC166	29099	HSPC166 protein	
R46859	0.000140203				
R88987	0.000166588	TTR	7276	transthyretin (prealbumin, amyloidosis type I)	carrier activity; extracellular space; retinol binding; steroid binding; thyroid hormone generation; thyroid hormone transporter activity; transport
H30513	0.000168118				

R32199	0.000177648				
R97023	0.000204063				
AA044052	0.000214526				
W16794	0.000221025	BIVM	54841	basic, immunoglobulin-like variable motif containing	
H17218	0.000223699	CALM2	805	calmodulin 2 (phosphorylase kinase, delta)	G-protein coupled receptor protein signaling pathway; calcium ion binding; cytoplasm; plasma membrane; protein binding
T84134	0.000228472	HMBS	3145	hydroxymethylbilane synthase	heme biosynthesis; hydroxymethylbilane synthase activity; lyase activity
H87044	0.000235822	TIMM22	29928	translocase of inner mitochondrial membrane 22 homolog (yeast)	inner membrane; integral to membrane; intracellular protein transport; mitochondrial inner membrane pre-sequence translocase complex; mitochondrion; protein translocase activity
R14705	0.000243616	FBXL2	25827	F-box and leucine-rich repeat protein 2	cytoplasm; protein binding; protein modification; proteolysis and peptidolysis; ubiquitin-protein ligase activity
AA031681	0.000245545				
W69443	0.000249658	HMGN1	3150	high-mobility group nucleosome binding domain 1	DNA binding; RNA polymerase II transcription factor activity; chromatin; positive transcription elongation factor activity
W32180	0.000251562				
R06754	0.000254895				
R66012	0.000286351				
R27906	0.000291				

	142				
H77950	0.000292038				
R56046	0.000313918	GNAZ	2781	guanine nucleotide binding protein (G protein), alpha z polypeptide	G-protein coupled receptor protein signaling pathway; GTP binding; endoplasmic reticulum; heterotrimeric G-protein GTPase activity; nuclear membrane; plasma membrane; receptor signaling protein activity; signal transduction
R81039	0.000324399	MFGE8	4240	milk fat globule-EGF factor 8 protein	cell adhesion; cell adhesion molecule activity; lipid particle; milk protein; oncogenesis
R53914	0.000336246	HARC	55664	Hsp90-associating relative of Cdc37	cytokinesis; regulation of cell cycle
R27994	0.000401721	LOC162427	162427	hypothetical protein LOC162427	
H52061	0.000413197	FLJ22313	64224	hypothetical protein FLJ22313	
H17731	0.000428912				
H68952	0.000434047	ITGA1	3672	integrin, alpha 1	cell adhesion receptor activity; cell-matrix adhesion; collagen binding; integral to membrane; integrin complex; integrin-mediated signaling pathway; magnesium ion binding; receptor activity
AA046698	0.000436088	KIAA1724	85465	KIAA1724 protein	phospholipid biosynthesis
T96360	0.000442922				
H84325	0.000463215	PBX3	5090	pre-B-cell leukemia transcription factor 3	DNA binding; anterior compartment specification; oncogenesis; posterior compartment specification

T95099	0.000466639	MT1F	4494	metallothionein 1F (functional)	biological_process unknown; cadmium ion binding; copper ion binding; cytoplasm; metal ion binding; zinc ion binding
R26644	0.000471842				
AA074208	0.000476658	NELL2	4753	NEL-like 2 (chicken)	calcium ion binding; cell adhesion; extracellular; structural molecule activity
H18471	0.000480051				
H53033	0.000483447	NUMB	8650	numb homolog (Drosophila)	integral to plasma membrane

Appendix I. Genes differentially regulated in the presence of both PTIO and SN50 with 15m concurrent MPP+ exposure.

GENBANK	SYMBOL	LOCUS LINK	GENE NAME	GENE ONTOLOGY
AA010141	SERPINH1	871	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	heat shock response
AA026902	FLJ11320	55343	GDP-fucose transporter 1	Golgi apparatus; integral to membrane; sugar porter activity; transport
AA028961				
AA031465	GEFT	115557	RAC/CDC42 exchange factor	
AA031859	TIMM13	26517	translocase of inner mitochondrial membrane 13 homolog (yeast)	hearing; mitochondrial inner membrane pre-sequence translocase complex; mitochondrial translocation; mitochondrion; protein targeting; protein translocase activity; zinc ion binding
AA034109	MINK	50488	misshapen/NIK-related kinase	ATP binding; cAMP-dependent protein kinase activity; development; protein amino acid phosphorylation; protein kinase CK2 activity; protein kinase cascade; protein serine/threonine kinase activity; response to stress; small GTPase regulatory/interacting protein activity; transferase activity
AA037284	APRT	353	adenine phosphoribosyltransferase	adenine phosphoribosyltransferase activity; adenine salvage pathway; nucleoside metabolism; transferase activity, transferring glycosyl groups
AA125808	CAPS	828	calcyphosine	calcium ion binding; intracellular signaling cascade
AA142924	DF	1675	D component of complement (adipsin)	chymotrypsin activity; complement activation, alternative pathway; complement factor D activity; hydrolase activity; proteolysis and peptidolysis; trypsin activity
AA152287	SLC35B2	347734	solute carrier family 35, member B2	copper ion binding; electron transport; electron transporter activity
H02590				
H09429				
H14566				
H26552	MGC5395	79026	hypothetical protein	intracellular signaling cascade

			MGC5395	
H28534	AQP1	358	aquaporin 1 (channel-forming integral protein, 28kDa)	excretion; integral to plasma membrane; transport; water transport; water transporter activity
H41330	LRRC2	79442	leucine-rich repeat-containing 2	
H45241	RPL41	6171	ribosomal protein L41	RNA binding; cytosolic large ribosomal subunit (sensu Eukarya); protein biosynthesis; structural constituent of ribosome
H47114				
H48570				
H48578				
H50015				
H50657				
H58461		339088	similar to My016 protein	
H59405	FLJ10298	54682	hypothetical protein FLJ10298	
H61842				
H63763				
H65775				
H65832				
H68885	TSSC3	7262	tumor suppressing subtransferable candidate 3	apoptosis; imprinting
H69845				
H83488				
H84657	GRWD	83743	glutamate rich WD repeat protein GRWD	
H85811	HIPK2	28996	homeodomain interacting protein kinase 2	nucleus; protein kinase activity; transcription co-repressor activity
H99202	MGC4126	84859	hypothetical protein MGC4126	
N/A1				
N23779	CD151	977	CD151 antigen	cell adhesion; integral to plasma membrane; membrane fraction
N29429	CGI-57	27013	hypothetical protein CGI-57	
N55283	KIAA0469	9903	KIAA0469 gene product	
N91376	KIAA0247	9766	KIAA0247 gene product	integral to membrane
N92911	DJ473B4	56180	hypothetical protein dJ473B4	structural molecule activity
R00907	PLEKHG1	57480	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	

R07137	HIC2	23119	hypermethylated in cancer 2	DNA binding; negative regulation of transcription, DNA-dependent; nucleus; protein C-terminus binding
R07186				
R09962	PMS2L6	5384	postmeiotic segregation increased 2-like 6	damaged DNA binding; mismatch repair; nucleus
R13021	FLJ10751	55222	hypothetical protein FLJ10751	
R14326	HERC1	8925	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1	ARF guanyl-nucleotide exchange factor activity; Golgi apparatus; catalytic activity; nonselective vesicle transport; ubiquitin cycle; ubiquitin-protein ligase activity
R14363	HDAC5	10014	histone deacetylase 5	chromatin modeling; chromatin silencing; cytoplasm; histone deacetylase activity; nucleus; regulation of transcription, DNA-dependent
R15267				
R20019				
R20373	TMP21	10972	transmembrane trafficking protein	ER to Golgi transport; Golgi apparatus; integral to plasma membrane; intracellular protein transport; membrane fraction; microsome; protein carrier activity; protein transporter activity
R21825				
R22402				
R23778	C7	730	complement component 7	complement activation, alternative pathway; complement activation, classical pathway; complement activity; cytolysis; immune response; integral to membrane; membrane attack complex; response to pathogenic bacteria
R26954	CTSD	1509	cathepsin D (lysosomal aspartyl protease)	cathepsin D activity; hydrolase activity; lysosome; pepsin A activity; proteolysis and peptidolysis
R34114				
R36086				
R47859	NPR1	4881	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	ATP binding; cGMP biosynthesis; guanylate cyclase activity; integral to membrane; intracellular signaling cascade; lyase activity; peptide receptor activity, G-protein coupled; protein amino acid phosphorylation; protein kinase activity; receptor activity; receptor guanylate cyclase activity; regulation of blood pressure

R55491				
R62213				
R83014				
R83247	GLB1	2720	galactosidase, beta 1	beta-galactosidase activity; lysosome
R87060	GGCX	2677	gamma-glutamyl carboxylase	blood coagulation; gamma-glutamyl carboxylase activity; integral to membrane; ligase activity; membrane fraction; protein modification
R87345	MGC2656	79414	hypothetical protein MGC2656	
R88895	MANBAL	63905	mannosidase, beta A, lysosomal-like	integral to membrane
R94499	GNB5	10681	guanine nucleotide binding protein (G protein), beta 5	
R98591				
T70299				
T79552				
T83013	HGD	3081	homogentisate 1,2-dioxygenase (homogentisate oxidase)	
T86338				
T92003	KIAA0342	9881	KIAA0342 gene product	DNA binding; membrane; nucleus; transport; transporter activity
T93785				
W68050	LGALS1	3956	lectin, galactoside-binding, soluble, 1 (galectin 1)	apoptosis; heterophilic cell adhesion; sugar binding
W72400	C12orf2	11228	chromosome 12 open reading frame 2	neuropeptide signaling pathway