

Identification of Parkinson's disease candidate genes

A Dissertation Presented

by

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Submitted to the Graduate School of the
University of Bahai's Institute for Higher Education in partial fulfillment
of the requirements for the degree of

BACCALAUREATE OF SCIENCE

September 2008

BIOLOGY DEPARTMENT

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IDENTIFICATION OF PARKINSON'S DISEASE CANDIDATE GENE

SEPTEMBER 2008

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TABLE OF CONTENTS :

LIST OF TABLES: VII

LIST OF FIGURES: VIII

ABSTRACT: 9

INTRODUCTION:..... 10

MATERIALS AND METHODS : 35

 DATABASE SEARCH: 35

 DATA VALIDATION: 35

 GENE LOCATIONS ON HUMAN GENOME AND MAPPING INTO THE PARK LOCI 36

RESULTS : 37

 TRANSCRIPTS COLLECTION AND PROCESSING: 37

 UNIFICATION OF GENES BASED ON MICE GENOME:..... 38

 VALIDATION OF RESULTS: 39

 CYTOGENIC LOCATIONS: 41

 CORRELATING GENES LOCATION WITH PREVIOUSLY RECORDED PARK LOCI 42

DISCUSSION AND FUTURE WORKS :..... 45

CONCLUSION : 50

REFERENCES : 51

APPENDICES:..... 64

LIST OF TABLES:

	Page
TABLE 1 . Representing PARK loci identified for PD so far	16
TABLE 2 : Candidate genes underlying PD disorder suggested by Gherbassi et al.	32
TABLE 3: The total number of genes within each study and the number of genes, located in PARK loci for each study	38
TABLE 4: Nominated genes identified by this study.....	44
TABLE 5 . Results of a study by Cantuti-Castelvetri et al. and compare it with nominated genes in this study	49

LIST OF FIGURES:

Figure 1 11

Figure 2..... 17

Figure 3..... 19

Figure 4.....21

Figure 5.....22

Figure 6.....23

Figure 7 24

Figure 8.....25

Figure 9.....29

Figure 10:..... 41

Figure 11 43

Figure 12..... 46

ABSTRACT:

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The main pathological characteristic of the disease is the degeneration of dopaminergic neurons in Substantia Nigra pars compacta (SNpc). Familial linkage studies have linked thirteen human chromosomal loci, called PARK, which are to the disease and nine out of hundreds of genes located in these loci have been identified as PD genes. Identification of responsible gene(s) within large number of genes residing in these loci requires multiple approaches, including screening methods to narrow down the number of genes. Since several of the identified PD genes are expressed in the SNpc, expression within the neurons of this area could be a suitable criteria to limit the number of genes in PARK loci. In this work I have used the combination of findings from six transcriptome analysis studies, carried out in mice and rats, studying the gene expression profile of midbrain dopaminergic neurons, in addition to the PD linkage studies to identify further candidate genes for PD association studies. Merging the two sets of studies, I identified 20 genes in different PARK loci, 9 of which are located in orphan PD loci and one has been previously identified as a gene responsible for PD. This list expands the number of candidate genes, which can be further analyzed in genetic association studies and provide cues into the molecular and cellular basis for degeneration of mesencephalic dopaminergic neurons in SNpc, during the course of PD.

INTRODUCTION:

Parkinson's disease(PD) is the second most common neurodegenerative disorder after Alzheimer disease(AD) affecting about 1 % of the individuals older than 50 which first described by James Parkinson as “Shaking Palsy “ in 1817 (Forno et al.,1996, Beal et al.,2000, Parkinson et al.,2002). Parkinsonism is a term to point out clinical manifestations of the PD. Resting tremor, dysautonomia, dementia, muscular rigidity, bradykinesia and postural instability are the most frequent indications of classical parkinsonism (Wijker et al., 1996).

Although the exact cause of PD is still unknown, selective degeneration and loss of mesencephalic dopaminergic neurons residing in substantia nigra pars compacta(SNpc) of midbrain is known as the pathological hallmark of the disease, which results in decrease of dopamine(DA) production and appearance of subsequent PD symptoms(Nussbaum and Polymeropoulos et al. , 1997). The neurons of SNpc are especially vulnerable to degeneration, and despite their evolutionary and genetic similarities, dopaminergic neurons of the neighboring population, ventral tegmentum area(VTA) are significantly less susceptible to cell death(Simon et al., 2003).

Another pathological feature of PD is presence of Lewy bodies(LB) and Lewy neurites(LN) diffusely scattered over the central nervous system based on stage of the disease as well as PD subtype (Lewy et al.,1939). LBs are spherical intracytoplasmic, proteinous, eosinophilic, detergent-insoluble inclusions with dense core surrounding by a halo and LNs are thread-like structures in neuronal perikarya. These two structures, which are frequently found in postmortem PD patient brains, first described by Friedrich Lewy in 1912 (Figure 1.) (Auluck et al., 2002, Nussbaum and Polymeropoulos et al., 1997, Shults et al, 2006).

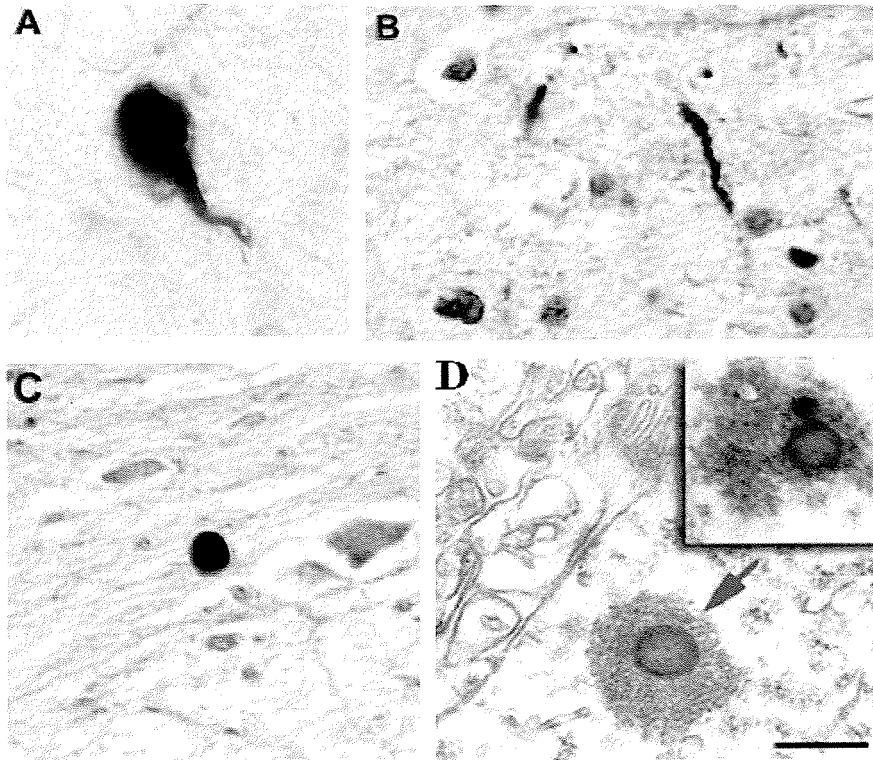


Figure 1. Cytoplasmic inclusions from human patients with PD (A, B and C) and rotenone infused rats (D). Lewy bodies (A, B and D) and Lewy neurites (C) in SNpc neurons of PD patients. These sections prepared from paraffin-embedded PD patient's brain immunopositive for molecular chaperon Hsp70 (A and B) and Hsp40 (C). Ultra structure analysis of inclusions show dense core with peripheral fibrillar elements (arrow). **Inset** an alpha-synuclein-positive inclusion as demonstrated by immuno-electron microscopy. Bar is 25 μm in (A), 32 μm in (B) and 50 μm in (C) 1 μm (adopted from (Auluck et al., 2002, Betarbet et al., 2000)).

Epidemiological studies suggest two kinds of PD; one familial which account for 10-15 % of all PD cases and the other sporadic or idiopathic with spontaneous onset in individuals. Despite their unequal incidence there is no clinical differences between familial and late onset PD (Hughes et al., 1992, Marder et al., 2003).

Investigation for getting better insight into PD pathogenesis process and underlying cause as well as its progression mechanism began. Of these attempts was detection of several PD-involved individuals within four generations in an afflicted kindred. These cases pursue same model with onset in their thirties that progress to death in 2 to 12 years (Spellman et al, 1962).

Another study on same family illustrated that individuals with affected first-degree relatives are two to three times more likely to become affected (Marder et al., 2003). Multiple other familial aggregation studies were performed and demonstrated high incidence of PD in some special families lead them to proposed hereditary factors as one of the causes of PD.(Tune et al., 1982, Autere et al., 2000, Rybicki et al., 1999).

Of the studies, planned to assess influence of hereditary factors in PD, is twin studies. A case report presented a pair of female monozygotic(MZ) twins with onset of PD at their thirties, provided more evidence for the genetic component of the disease(Kessler et al., 1978). On the contrary, another study noted no concordance with PD in twelve MZ twins(Duvoisin et al., 1981). In support of the latter was an examination on sixty two twins(19 dizygotic and 43 MZ) that discovered only one twin pair concordance to the disease and concluded that frequency of PD occurrence in twins is lesser than incidence of the disease(Ward et al., 1983). Low concordance between 268 examined twins provided more evidence to underestimate role of genetic factors in PD and let them to state that there is no genetic component associated with PD cases with the age of onset of more than 50 years, and suggested minimal role in those PD cases(Tanner et al., 1999).

The initial reports for the contribution of non-genetic causes to the disease and the role of extrinsic factors suggested viral infection as an important contribution factor, due to a significant increase in the number of PD cases after pandemic infection by influenza virus(Poskanzer and Schwab et al, 1961). Other environmental factors were also shown to have direct correlation with PD. Dieldrin is a long lasting, highly lipophilic organochlorine synthetic compound which used to be an effective pesticide and good alternative for DDT in 1940s. The pesticide accumulates in lipophilic organs of human body, specially in CNS with a half life of about 300 days(Brock et al., 1998, Kanthasamy et al., 2005). Association between time and dosage of exposure to this pesticide with decrease in dopamine and Parkinson disease were confirmed by several studies (Corrigan et al., 2000, Fleming et al., 1994, Sanchez-Ramos et al., 1998).

Another evidence for the role of extrinsic factors in PD came from observation of PD outbreak in young drug abusers. 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine(MPTP or Meperidine) is a synthetic opiates which induced parkinsonism by selective degeneration of DA neurons of the SNpc(Langston et al., 1983). MPTP is also shown to produce proteinous intracellular structures resembling LB in aged nonhuman primates(Forno et al., 1988).

Similar effect is seen for insecticides, herbicides and fungicides such as Rotenone, Paraquate and Manganese ethylenepistithiocarbamate(maneb) respectively. Injection of Rotenone to rodents either intravenously or subcutaneously induced parkinsonism by degeneration of DA neurons (Betarbet et al., 2000). Maneb and paraquate respectively illustrate DA neuronal loss and parkinsonism in animal models, further supporting role of environmental factors in PD (Meredith et al., 2004). More investigations reported interference of these compounds with mitochondrial respiration by acting as inhibitors for complex I electron transport chain(ETC). In contrast to MPTP and paraquate, Rotenone does not concentrate in dopaminergic neurons suggesting sensitivity DA neurons sensitivity to complex I impairment in CNS neurons(Sherer et al., 2003)

These findings supported multifactorial cause of the disease(Le Couteur et al., 2002) lead researchers to a consensus about gene-environment interactions in PD occurrence rather than exclusive genetic or extrinsic factors. Meanwhile an evaluation on PD cases divided them into four etiological categories : postencephalitic, idiopathic, genetic, and symptomatic(Barbeau and Pourcher et al, 1982) .

For several years before Lewy discovery, Parkinsonism was the only way of PD diagnosis however further investigation indicates that parkinsonism is also produce in other diseases like Progressive supranuclear palsy, frontotemporal dementia, autosomal Segawa syndrome, X-linked recessive Filipino type of dystonia and cerebrovascular disease(Forno et al, 1996). A clever research performed to evaluate accuracy of PD diagnosis based solely on its clinical manifestations(parkinsonism) found 25% misinterpretation of individuals as PD by neurologists(Hughes et al., 1992).

In searching for suitable diagnostic evidence, detection of LBs and LNs in brains of afflicted individuals suggested as unique criterion however later studies demonstrate presence of these structures not only in PD but also in various neurological disorder such as; Lewy body variant AD, LB dementia(LBD), Down's

syndrome, neurodegeneration with brain iron accumulation type 1 (also known as Hallervorden–Spatz disease), progressive autonomic failure, rapid eye movement sleep disorder, Gaucher's disease and Pick's disease (Shults et al, 2006). Moreover some PD subtypes do not show LB formation as well. Of these are juvenile autosomal recessive parkinsonism, the rapidly progressive autosomal dominant parkinsonism associated with dementia, dystonia and disinhibition and X-linked dystonia-parkinsonism(Shults et al, 2006). Thus, although LB and LN could be important in diagnosis of PD, presence of these structures *pre se* do not meant PD and should be correlated with pertinent clinical examinations which include not only evaluation of parkinsonism, but also other clinical symptoms like gastrointestinal motility measurements as well as L-DOPA responsiveness of the disease.

Despite these deficiencies in accurate PD diagnosis some researchers tried to get more insight about the disease and began familial as well as segregation analysis studies which mainly looked at PD as a hereditary disorder. A segregation analysis performed on 948 ascertained PD cases collected from three medical schools with mean age of onset about 57.7 years showed similar proportion of affected individuals across three sets of data. The results follow same pattern of 60.4% male and 39.6% female. Among these, 4.7% were fathers, 2.9% brothers, 6.6% mothers and 3.2% sisters. From these findings they concluded presence of a rare mendelian mode of inheritance in PD cases. They also interpreted the rare condition as interference of gene-environment interaction (Maher et al., 2002). This finding also presented more incidence of the disease among men.

Comparing relative risk among three groups consisting of patients with early onset (>50, 221 individuals), late-onset (< 50, 266 individuals) and control group (409 individuals) and considering several factors including sex, age, education, duration of the disease and ethnicity suggested an autosomal recessive model of inheritance in early onset PD and increased risk of PD in whites in comparison with Hispanic relatives(Marder et al., 2003). Although these models of inheritance were mainly found in early-onset form of PD cases, genetic contributions are equally important in both early and late-onset types and as

suggested by its author they may differ in genes abnormalities and models of inheritance (Marder et al., 2003).

By recent advanced imaging techniques, positron emission tomography, significant higher concordance of PD cases in eighteen MZ in comparison with sixteen DZ twins was found demonstrating substantial role of hereditary factors in sporadic PD (Piccini et al., 1999). This finding demonstrated that misleading results in twin studies was due to lack of accurate diagnostic criteria that bring about hampering genetic approaches for decades.

Familial aggregation studies (as some described above) could identify multiple PD subtypes. Thus these kinds of studies not only demonstrates importance of genetic factors, but also provided well-documented families with special phenotypes on which DNA linkage studies could be carried out to identify genetic anomalies leading to PD. Since 1997 in which linkage studies began to develop multiple attempts on different PD subtypes populations to identify linkage of these individuals phenotypes with any chromosomal location have been done and based on current understanding thirteen specific regions on different chromosomes reported to have linkage to PD. These regions are commonly called PARK loci (Table 1).

TABLE 1 . Representing PARK loci identified for PD so far

Loci	Gene name	Cytogenic location	Inheritance model	Probable functions
PARK 1	SNCA	4q21.1-4q21.3	AD	LB constituent, Presynaptic protein
PARK 2	PARKIN	6q25.3-q26	AR	Ubiquitin E3 ligase
PARK 3	Unknown	2p13.3-2p13.1	AD	Unknown
PARK 4	Unknown	4p15.33-4p15.1	AD	Unknown
PARK 5	UCH-L1	4p14	AD	Ubiquitin C terminal hydrolase
PARK 6	PINK 1	1p36.33-1p35.1	AR	MT kinase
PARK 7	DJ-1	1p36.23-1p36.22	AR	Chaperone, Antioxidant
PARK 8	LRRK2	12p11.2-12q13.13	AD	Mixed lineage kinase
PARK 9	ATP13A2	1p36.33-1p36.11	AR	Unknown
PARK 10	Unknown	1p33-1p32.2	AD	Unknown
PARK 11	Unknown	2q36.1-2q37.3	AD	Unknown
PARK 12	Unknown	Xq21-q25	Unknown	Unknown
PARK 13	HTRA2	2p13.1-2p11.2	Unknown	MT serine protease

AD ; Autosomal dominant, AR : Autosomal recessive (Thomas and Beal, 2007, Gherbassi et al., 2007)

The first PARK locus reported from a DNA linkage within a large kindred of pathologically confirmed, autosomal dominantly inherited PD Italian family with mean age of onset about 46 ± 13 (Polymeropoulos et al., 1997). They employed 140 gene markers with average spacing ~20 cM and found only one linkage at cytogenic location of 4q21.1-4q21.3. This locus later called PARK 1.

One year later he identified a missense mutation in alpha synuclein gene Ala53→Thr (A53T) in Italian-American Greek kindred at position 209 resulting from G to A transition (Polymeropoulos et al., 1997). Later, two other missense mutations, A30P and E46K as well as duplication and triplication of this area were also reported (Fuchs et al., 2007, Farrer et al., 2004).

Two latter findings suggested that increased amount of alpha-synuclein (SNCA) as a result of duplication or triplication of the alpha synuclein would cause the disease in that PD subtype(Singleton et al., 2003). Alpha synuclein belongs to highly conserved gene family indicating their critical role in cells consisting of three protein subtype of alpha, beta and gamma synuclein. Alpha-syn protein in mice, consists of seven exons, five of which are protein coding. This intrinsically unfolded protein seems to participate in synaptosomal preparation, neurotransmitter recycling in presynaptic neurons and neuronal plasticity.

Structurally alpha-syn consists of three compartments(Figure 2) ; one end of the protein is occupied by amphipathic N-terminal region which made near two third of the molecule(89 from 140 amino acid) and contains some motifs mediating binding of this protein to vesicles. An aciditic C-terminal region which seems to play important role in SNCA aggregation is placed on another pole of this protein. Between these two, a hydrophobic region locates containing non-amyloid- β component(NAC) domain residing between 61-95 residues as depicted below. The latter domain of SNCA demonstrated to have aggregation propensity of these proteins to both fibrillar and glandular forms, since over-expression of this protein lacking 71-82 residue (A part in NAC) in flies show no aggregation (Giasson and Lee, 2001)

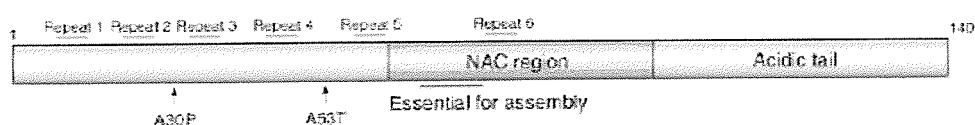


Figure 2. Schematic diagram showing SNCA structure (Giasson and Lee et al., 2001)

This protein in addition to ubiquitin are also shown to be the main components of LB, however other constituents such as neurofilaments are also exist in them.

SNCA expression level measurements in neurons within which LBs are usually found in PD patients and comparing it to other brain regions

demonstrated substantial SNCA expression for aggregation in different brain neurons. This finding suggests that presence of this protein *per se* do not lead to their aggregation and other factors like suitable environment and/or other complementary factors are necessary in LB formation (Solano et al., 2000).

Alpha synuclein knockout mice provide useful tools to explore its underlying molecular pathways and interacting proteins which leads to PD and searching for factors that promote SNCA aggregation and underlying mechanism accelerated. Investigations in these mice revealed role of alpha synuclein in genesis, localization and maintenance of at least some of the reserve or resting pool presynaptic vesicles (Cabin et al., 2002).

Some studies focus on Alpha synuclein modifications and some other evaluate effects of other factors. As mentioned one of the major components of LB is SNCA. More detailed investigations show presence of modified form of this protein in LB mainly in oxidized and/or phosphorylated forms. Phosphorylation at Ser-129 residue of Alpha syn can lead to four fold increase in its insolubility. About 4% of Alpha syn are phosphorylated at Ser-129 in normal physiological condition however this could arise to 89 % in Alpha syn constitute LB (Shults et al., 2006). Role of G protein coupled receptor kinases and casein kinase 1 and 2 as two major type of kinases responsible for phosphorylation of Ser-129 residue of alpha syn are also evaluated (Beal et al, 2000) .

A study replaced 129-residue by alanin and aspartate to prevent and mimic phosphorylation of this residue respectively to detect toxicity of phosphorylated alpha syn. The study revealed that SN neurons in unphosphorylated (alanin replacement) alpha syn was more vulnerable to produce LB and neuronal loss was completely suppressed. However in phosphorylated form (aspartate replacement) alpha syn shows significant toxicity suggesting alpha syn toxicity in soluble form and LB formation as an protective strategy of the cell. They also hypothesized that phosphorylation at 129 residue favors maintenance of this protein in soluble form (perhaps toxic) leading to degeneration of SN neurons (Chen and Feany et al., 2005) . Supporting these findings was a study which showed that over-expression of G protein coupled receptor kinase enhances alpha synuclein toxicity (Beal et al., 2000).

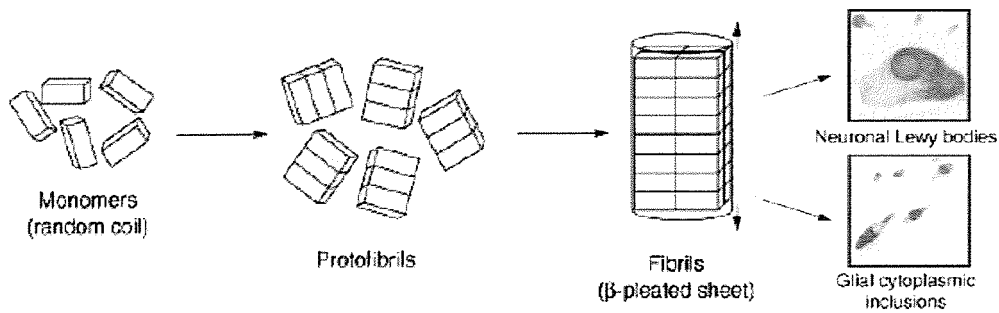


Figure 3. A model demonstrating alpha-synuclein monomers assembly into both LBs and LNs . In this process protofibrils are more soluble than fibrillar forms (Giasson and Lee et al., 2001)

Wild type SNCA in SN neurons could aggregate under oxidative stress resulted from mitochondrial complex I dysfunction or treatment of cells with MPTP or any other MT complex I inhibitors. These data could suggests that mitochondrial dysfunction produce suitable environment for alpha syn aggregation and LB formation(Giasson and Lee et al., 2001). Another approach for understanding SNCA polymerization and LB formation mechanism come from the observation of presence of *tau*, a microtubule associated protein, inclusions in SN neurons of both AD and mutant (A53T) SNCA induced PD (Giasson and Lee et al., 2001).

Several examinations demonstrated that both *tau* and SNCA mutually promote their polymerization initiation however in other stages, elongation and termination, processes accomplished separately to form homopolymers instead of heteropolymers(Giasson and Lee et al., 2001). Although multiple factors are shown to enhance aggregation of these proteins, still number of factor(s) remains to be discovered .

It is still not clear that accumulation of missfolded proteins which from LBs in PD as well as other neurodegenerative disorders are neurotoxic or they are protective response to other degenerative factor(s). In addition to Chen and feany who hypothesize accumulation of phosphorylated alpha syn in LB could reduce SNCA toxicity, some pharmacological compounds demonstrated to produce inclusions as protective agents against SNCA toxicity. Contrary to these

results is the observation of reduced injury of SN neurons after paraquat treatment in transgenic mice over expressing alpha syn(Thomas and Beal et al. , 2007).

Alpha syn polymerize to form soluble protofibrils and more polymerized amyloid fibrillar aggregates. An intermediary, spherically arranged, β sheet structure (protofibril) in its polymerization which later fibrillated to form fibrillar SNCA occur in SN neurons show toxicity for neurons. Supporting this notion was observation of two distinct forms of SNCA ; primarily formed spherical alpha syn inclusions called protofibrils and secondarily formed larger inclusions in two kind of cultured cells(Thomas and Beal et al. , 2007, Shults et al., 2006) .

Lots of attempts were made to identify mechanisms by which SNCA aggregates could injure neuronal cells. Based on our current understanding some mechanisms have been proposed. Of these are protein aggregation, impaired protein degradation and mitochondrial dysfunction and/or oxidative stress. These integrated mechanisms, accompanying with other intrinsic and extrinsic factors make these injurious pathways more complex.

Complex I dysfunction shows to interfere with MT outer membrane permeability leading to imbalance of electrochemical gradient which trigger cascades of events which finally leads to increased nitrating agents, proteasome dysfunction and apoptosis.

Abnormally processed alpha synuclein, either due to mutations in SNCA or abnormal post-translational modifications, could accumulate and aggregate within cells lead to neurodegeneration through different mechanisms. This large protein occlusion could change cell shape and interfere with normal trafficking in cells leading to cell death. Of these kind of interruption is blocking vesicular trafficking from endoplasmic reticulum to Golgi apparatus resulting in ER stress (Cooper et al., 2006).

Another way by which SNCA aggregate could injure the cells is that accumulation of these structures in neuronal cells can impair systems responsible for clearance of misfolded and/or abnormally processed proteins from the cells (e.g. proteasomes and lysosomes systems). Over expression of mutant SNCA, A53T and A30P, shows disruption of vesicular PH and its normal function which enhance oxidized catechol metabolite toxicity making SN

neurons more vulnerable to death since high level of cathecole are present in SN cells.

From MPTP and other inhibitors of MT complex I findings role of MT in producing oxidative stress is obvious. In addition several reviews demonstrated role of energy in pathogenesis of several neurodegenerative disorders including PD and demonstrated MT role in apoptosis (Beal et al., 2000)

Mitochondria surrounded by outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) and intermembrane space (IMS) which locates between these two contains multiple proapoptotic and oxidative phosphorylation required enzymes. In addition to several nuclear and mitochondrial encoded proteins (~1,500 different proteins) matrix is also present surrounded by IMM. This organelle participate in both ATP production, through electron transport chain (ETC) made up of five complexes, and apoptosis initiation by releasing proapoptotic molecules into cytosol (Figure 5).

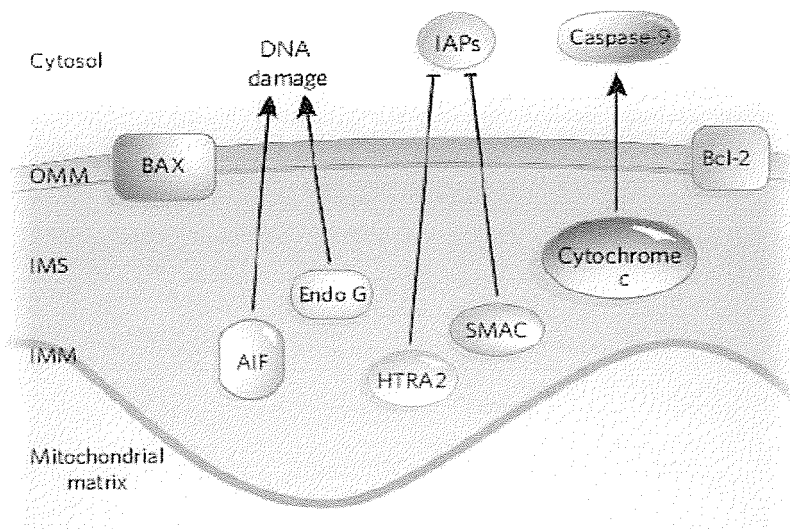


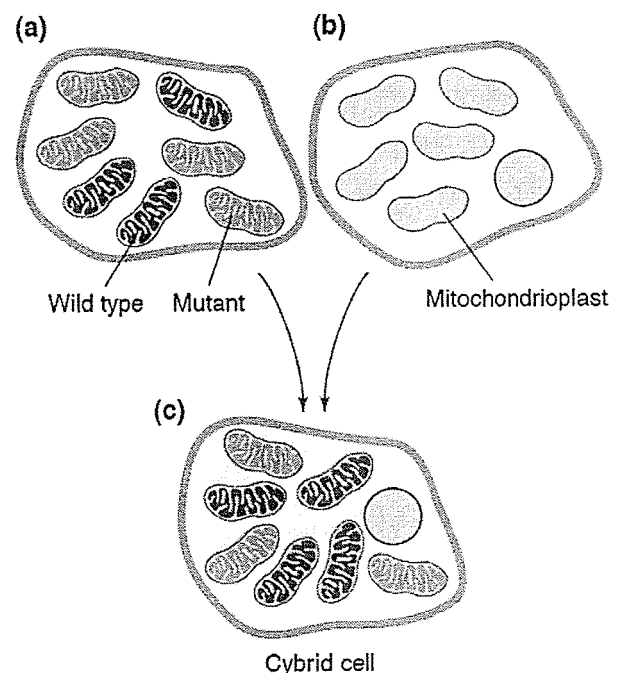
Figure 4. Mitochondrial role in apoptosis. Several intermembrane space (IMS) proteins are pro-apoptotic if released into the cytosol. Cytochrome c activates caspase-9. SMAC (second mitochondrial activator of caspases) and HTRA2 inhibit cytosolic inhibitor of apoptosis proteins (IAPs). HTRA2 is a serine protease that might function to remove denatured proteins within mitochondria, but degrades IAPs when released from mitochondria. Inhibiting HTRA2's normal quality control function or enhancing its IAP-degrading activity could both promote cell death.

Apoptosis inhibitor factor (AIF) and endonuclease G (endo G) translocate to the nucleus and induce chromatin condensation and DNA fragmentation. Release of these proteins into the cell is modulated by recruitment of BAX (which is proapoptotic) or Bcl-2 (anti-apoptotic) to the outer mitochondrial membrane (OMM) (Lin and Beal et al., 2006).

OMM is permeable to small molecules, however fibrillar aggregation of SNCA can produce pores ranges from 1 to 3 nm on this membrane, which could pass charged molecules that interrupt normal MT function and cause their fission. Some other molecules like Bax could insert themselves in microfilaments and widened pore diameter large enough to leak proapoptotic molecules into cytosol, which could initiate apoptosis through different cascades. The pores not only form on OMM but also can be made on vesicles and proteasomes, interfering in their normal physiological function (Lin and Beal et al., 2006).

The evidence that mitochondria complex I inhibitors could leads to PD, and its role in apoptosis and ATP production, candidate this organelle to play important role in PD occurrence. Strong support to this notion come from Cybrid cell studies demonstrating MtDNA encoded proteins defects as a reason for PD pathogenesis (Figure 5).

Figure 5. Cybrids for investigating mitochondrial DNA (mtDNA) defects. Although mitochondria are strongly implicated in neurodegenerative diseases, new techniques are needed to investigate their role. A novel technique pioneered by King and Attardi is to transfer mitochondria from living cells (a) to mtDNA-deficient cell lines [rho0 cells; (b)]. Cell lines can be depleted of mtDNA by exposing them to low concentrations of ethidium bromide, which inhibits replication of mitochondrial but not nuclear DNA. Exposed cells lose their mtDNA and assume an anaerobic phenotype. Cybrids are cells formed by fusing mitochondria from a patient's platelets or other tissues with the rho0 cells (c). If defects in oxidative phosphorylation are found in the cybrids they are attributable to



alterations in a patient's mtDNA, as the patient's mitochondria now function in the presence of a different nuclear genome. Abnormal mitochondrial function has been reported in cybrids made from individuals with amyotrophic lateral sclerosis, PD and AD (adopted from Beal et al., 2000) .

As the result of cell respiration reactive oxygen species(ROS) are produced which are toxic to both MT and cells containing them. These toxic components are removed from the cell by ROS-scavenging enzymes like MnSOD, MRSA(methionine sulfoxide reductase A) and catalase. Any factor that derange this delicate balance would cause cell degeneration (Figure 6).

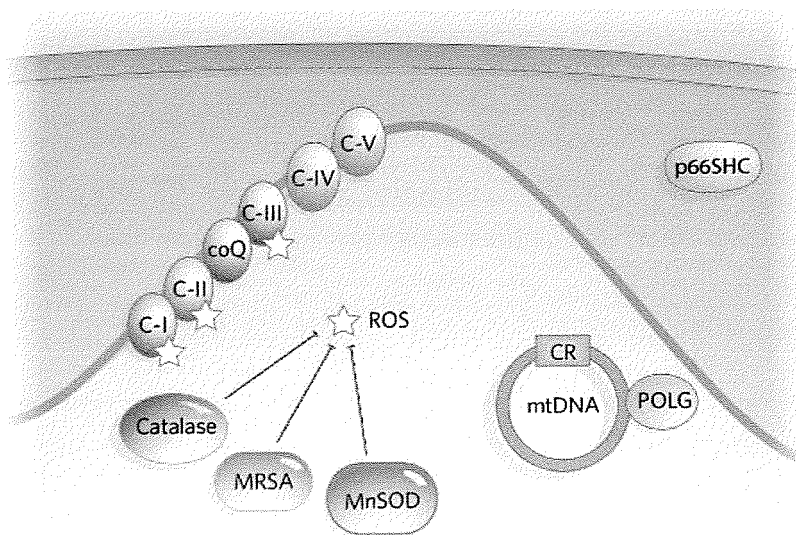


Figure 6: Mitochondrial DNA(mtDNA) mutations and oxidative stress may contribute to ageing. Somatic mutations accumulate in mtDNA with age. Inducing mtDNA mutations by disabling the proofreading activity of mtDNA POLG accelerates ageing-related pathology in transgenic mice. Certain mtDNA polymorphisms are associated with increased longevity, possibly by reducing membrane potential and decreasing the generation of reactive oxygen species (ROS, white stars). Overexpression of ROS-scavenging enzymes manganese superoxide dismutase (MnSOD), methionine sulfoxide reductase A (MSRA) or catalase within mitochondria prolongs lifespan. Knockout of p66SHC, a protein that promotes ROS generation and mitochondrial apoptosis, also prolongs lifespan. Complex IV (C-IV) and complex V activities decline with ageing, and knockdown of complex V activity causes oxidative damage to nuclear DNA, which probably results in decreased gene expression with ageing (Lin and Beal et al., 2006).

Mitochondrial scavenging enzymes role in ROS neutralization attracts some researchers interests as possible cause of PD through malfunction these enzymes which leads to increased ROS destructive actions and subsequent neuronal loss. A survey which primarily performed to detect gene abnormality in MnSOD gene reported second locus which links to PD. By examining 50 members of 13 families with mean age of onset about 24 years, although they failed to prove their hypothesis, they reported a locus in long arm of 6th chromosome which links to unique PD subtype, Autosomal Recessive juvenile parkinsonism (ARJP). This region is commonly called PARK 2. Specific feature of this PD subtype is absence of LB in afflicted individual's brain as well as low age of onset, before fourth decades of life (Matsumine et al., 1997). Moreover they hypothesized that mutation in either MnSOD2 gene-related area (e.g. regulatory regions) and/or an undetermined gene adjacent to MnSOD in that region may cause PD (Matsumine et al., 1997).

Further investigations on PARK2 chromosomal location shows several mutations in a gene called *PARKIN* (6q25.3-q26), which leads to its loss of function and ARJP (Lucking et al., 2001, Hoenicka et al., 2002). Structurally, PARKIN consists of 465 amino acids with a ubiquitin-like N-terminal domain, a central linker domain and a C-terminal region consisting of two RING finger motifs separated by an IBR (Figure 7).

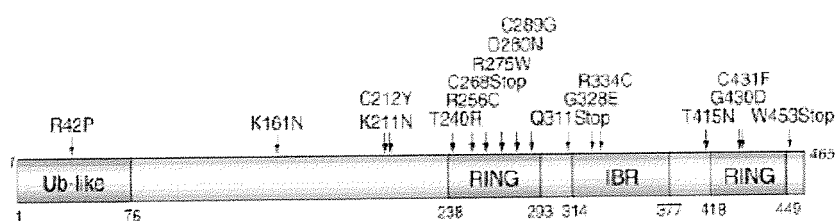


Figure 7. Schematic diagram showing several domains of PARKIN . Parkin consists of several domains; an amino-terminal ubiquitin-like domain (Ub-like) and two carboxy-terminal RING finger domains separated by an IBR. The location of disease-related point mutations resulting in amino acid substitutions or protein truncation is depicted. Additional frameshift and deletion mutants have been described (Giasson and Lee et al., 2001).

Several studies suggest a neuroprotective role PARKIN, against different types of neuronal insults. PARKIN is associated with outer mitochondrial membrane, which further protects it from swelling. This protein, like other RING finger containing proteins, acts as an E3 ubiquitin protein ligase, targeting misfolded(oxidatively damaged) proteins to ubiquitin-dependent proteasome pathway degradation (Darios et al., 2003) (Figure 8).

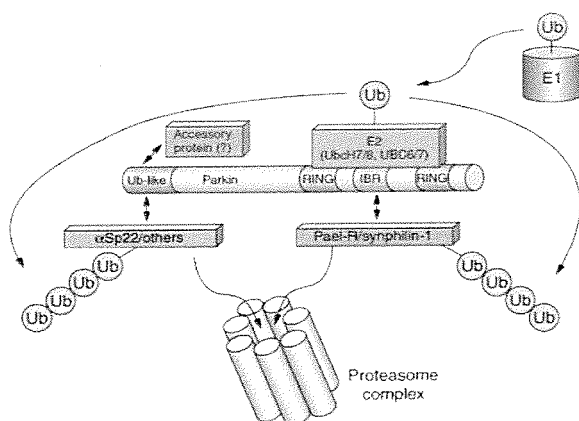


Figure 8. Involvement of Parkin in the ubiquitin-proteasome pathway.

Parkin (E3) serves as a link between the substrate for ubiquitination and E2s in which

bind to RING finger region, Alternative regions in parkin may recognize different substrates. The ubiquitin-like region is either required for recruitment of an accessory protein or the binding of some substrates that may include α Sp22. Peal-R and synphilin-1 interacts with RING finger region in but synphilin-1 preferentially binds to the IBR region and the second RING finger domain. Ubiquitin moieties are transferred from E1 to E2 and finally to the substrate, which becomes a target for degradation by the proteasome (Giasson and Lee et al., 2001) .

Soon after determination of these two PARK loci another locus was identified in a familial parkinsonism which has much more similarities with sporadic PD with age of onset about 59 years. By measuring logarithm of odds's(LOD) score in different chromosomal locations of PD patient's between 6 European families an specific region(2p13.3-2p13.1) with high LOD score indicating its linkage to the disease have been found(Gasser et al., 1998). Further investigation on this area, so called PARK 3, by constructing 17 micrsatellite, narrowed down the PARK 3 to a 2.5 Mb distance, however despite sequencing 14 known genes in that region from at least 2 patients, they failed to show any pathogenic mutation (West et al., 2005). Several attempts in this region have done to identify responsible gene however still no gene residing in that area

demonstrated to cause PD. One attempt in this region result in detection of three haplotype in sepiapterin reductase(7,8-dihydrobiopterin: NADP+ oxidoreductase) (*SPR*) gene. Individuals which carries one of these haplotypes show about 7.4-year younger mean age at onset in comparison with two other haplotypes in that region. From these findings authors concluded a possible role of that haplotype, harboring SPR gene, in modifying the PD age of onset (Karamohamed et al., 2003). In addition to SPR, other genes including transforming growth factor- α (TGFA), cytochrome P450 retinoid metabolizing protein (P450RAI-2), putative N-acetyltransferase Camello 2 (CML2) and a number of as-yet-undiscovered genes are predicted in this region, which requires further investigation (DeStefano et al., 2002).

A familial aggregation study on a Swedish American descent family with autosomal dominant early onset parkinsonism with onset on their 30s have identified in that PD subtype. Investigation in this family also suggests a duplication or triplication alpha syn as possible cause of that phenotype (Farrer et al., 2004). Genomewide search on this subtype demonstrated presence of 26 cM distance on chromosome 4q with high LOD score of 3.5 containing a haplotype cosegregating with the disease . This region is called PARK 4 and extends from 4q21 to 4q23(Singleton et al., 2003).

Another PD subtype with autosomal dominant transmission, early weight loss and Intellectual dysfunction, which begins with subjective memory loss and objective visuospatial dysfunction has reported in two previously described kindred (Gwinn-Hardy et al., 2000, Waters and Miller, 1994, Muentner et al., 1998). A genetic linkage study performed a genome-wide screen by using more than 300 markers in 1998 on those two families and identified linkage between postural tremor and 4p15.33-4p15.1 chromosomal location and called this region as PARK 5 (Farrer et al., 1999).

Responsible gene in that area which is another constituents of LB is ubiquitin C-terminal hydrolase L1(UCHL1). This protein contains 212 amino acids and exclusively find in various brain regions, particularly in SNpc, and genital gonads(tests/ovaries). UCHL1 gene contains 9 exons, spanning 10 Kb with CG-rich content between exon 1 and 3 (Day et al., 1990, Leroy et al., 1998). Hydrolysis as well as dimerization-dependent ubiquityl ligase activities of this

protein and association of it with monoubiquitin to increase ubiquitin half-life has been reported. Association between UCHL1 expression and ubiquitin availability further demonstrate the latter UCHL1 role. Increased expression of UCHL1 in both gad (gracile axonal dystrophy) mice and cell culture show increased amount of ubiquitin monomers and vice versa (Osaka et al., 2003). This protein also causes SNCA aggregation in cultured cells (Liu et al., 2002)

Another PD subtype, with similar symptoms to whom carries PARKIN-mutated gene have reported in so called Marsala kindred, but in contrast to them they did not carry any mutation in PARKIN (Albanese et al., 2005). By examination of 122 members from 30 families and excluding mutations in PARKIN new linkage study could found 4 affected individuals in both sexes. By employing 400 microsatellite markers they could find one region with maximum 4.01 LOD score on short arm of chromosome 1 (1p35-36) which called PARK 6 (Valente et al., 2001)

Several genes including those of homeobox genes, endothelin-converting enzyme 1, G protein coupled receptor in addition to PINK 1 (PTEN-induced kinase 1) resides in this region (Valente et al., 2001). More genetic studies in this area revealed 2 homozygous mutations (one nonsense and one missense mutation) in PINK 1 gene kinase domain (Valente et al., 2004). Several other heterozygous and homozygous mutations also were later reported (Hatano et al., 2004, Klein et al., 2005, Leutenegger et al., 2006). PINK1 is a mitochondrially located protein with protective effect which its loss of function due to mutations seems to increase cells vulnerability to cellular stresses (Valente et al., 2004).

Another research on PD cases was made on a genetically isolated community from southwestern Netherlands. Several gene markers for mapping the genome of that patient were used and specific area on chromosome one (1p36) was shown to link between PD cases. They also investigate whether this region is linked to PARK 6 and found more than 25 cM distance between centromeric PARK 6 and newly found PARK 7 (van Duijn et al., 2001).

Further attempts in this locus resulted in detection of two mutations in DJ1 gene (Bonifati et al., 2003). Several other mutations including exonic deletions as well as homozygous and heterozygous point mutations (L166P, M26I and E64D) were later detected in this gene leading to DJ1 loss of function (Abou-Sleiman et

al., 2003, Bandopadhyay et al., 2004). DJ-1 is a homodimeric protein, consisting of 189 amino acids, which belongs to highly conserved DJ-1/Thi/Pfpl protein super family. This protein, similar to PINK1, is localized inside mitochondria however, in contrast to it, expresses not only in the brain but also in other tissue. DJ1 initially was known to play role as an oncogene with important role in maturation and fertilization of sperm, but further exploration shows its diverse roles as an antioxidant, transcriptional co-activator and modulator of chaperone activity.

Also multiple protective effects of this protein have demonstrated (Abou-Sleiman et al., 2003). Over-expression of WT DJ1 in both cell culture and DA neurons in vivo show a protective effect against oxidative stress-induced toxicity. Oxidative stress shows to oxidize cysteine at position 106, which can be converted to cysteine sulfinic acid. This self oxidation ability seems to cause H₂O₂ elimination and acts as ROS scavenging enzyme (Thomas and Beal, 2007). Neurons in DJ1 knockout mice reported to be more vulnerable to MPTP-induced toxicity through an unknown mechanism. These mice develop dopamine production dysfunction without neuronal loss (Abou-Sleiman et al., 2003, Thomas and Beal et al., 2007). DJ-1 inhibits SNCA aggregation and has a neuroprotective effect in association with PARKIN (Beal, 2000).

A familial aggregation reported slow growing type of parkinsonism in a large Japanese family characterized by autosomal dominant mode of inheritance, variable age of onset (mainly in their 60s), laterality of parkinsonism, bradykinesia, rigidity, resting tremor, postural instability, neuronal loss and gliosis in the substantia nigra with excellent response to low doses of L-DOPA and without any cognitive decline even after long time affliction (Hasegawa and Kowa et al. 1997). A genome-wide linkage analysis on this Japanese family resulted in discovery of another chromosomal location, so called PARK 8 (12p11.2-q13.1), which links to PD (Funayama et al., 2002). Attempts to detect responsible gene(s) in this region began and several mutations in leucine rich repeat kinase 2 (LRRK2, also called Dardarin) gene were detected.

LRRK2 is a 286 kDa, multidomain protein consisting of 51 exons encoding 2527 amino acid which could be found in a variety of species from metazoans to human with diverse functions including tumor suppression. This protein belongs

to ROCO protein family that includes a Rho/Ras-like GTPase domain, a protein kinase domain of the MAPKKK family, a WD40-repeat and a leucine-rich repeat domains. An additional domain C-terminal to the GTPase domain, termed COR (for carboxy-terminal of Roc), is also present with unknown function (Gasser et al., 1998) Figure 9. Although the exact physiological function of this protein is still unknown, presence of multifunctional domains suggests its role in various functions. Of approaches to identify LRRK2 function is measuring localization of this protein within cells, in order to figure out its function. Series of studies performed in both cell culture and *in vivo* demonstrate high expression level of this protein in midbrain structures including dopaminergic neurons.

LRRK2 localizes in cytoplasm and mainly found in association with Golgi apparatus as well as synaptic vesicles lysosomes, plasma and outer mitochondrial membranes.

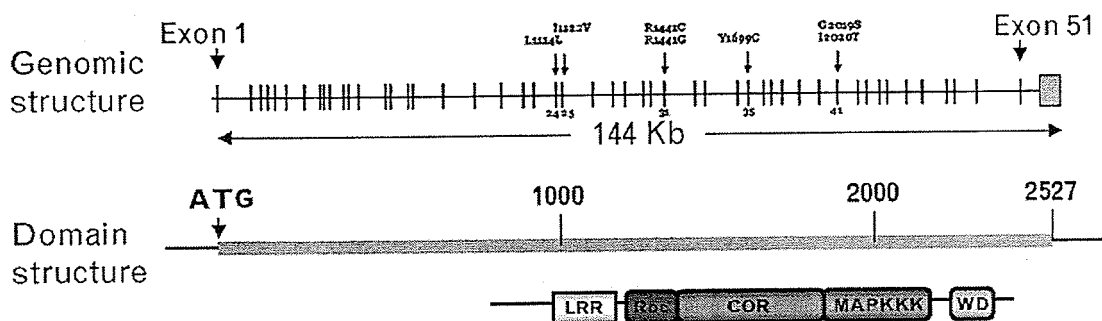


Figure 9, Schematic diagram depicting LRRK2 structure and mutations in PD .

LRRK2 spans 144 Kb and contains 51 exon . LRR, leucine rich repeat ; Roc, Ras of complex proteins ; COR, C-terminal of Roc ; MAPKKK, mitogen activated kinase kinase kinase ; WD, B-propeller (Gasser et al., 1998).

Until now several missense mutations have identified, of which the G2019S is the most prevalent and important, which account for ~5-6 % of all dominantly inherited parkinsonism cases as well as ~1-2 % of idiopathic PD demonstrating the important role of this gene in PD (Gasser et al., 1998). The latter accompanied by nearby I2020T mutation is located in MAPKKK domain which increase kinase activity of this domain (gain of function mutation). Although

the mechanism by which increased kinase activity of MAPKKK affect neuronal cells still unknown, there is growing evidence that increased kinase activity caused by mutations in that domain reduce cells viability via apoptosis leading to neurodegeneration. GTPase activity of ROC domain seems to regulate kinase activity of MAPKKK since mutations (R1441C and T1348N) in GTPase of ROC domain disrupt GTP binding and hydrolysis, abolishing kinase activity (Ross and Farrer et al. , 2005, Gasser et al., 1998).

A familial aggregation study in a Jordanian family revealed a syndrome which primarily called Kufor-Rakeb syndrome, indicating the place where the cases gathered. More evaluation in this disease demonstrated that it is another PD subtype which mostly resemble idiopathic one(Najim al-Din et al., 1994). Further exploration through genetic studies on that PD cases for mapping their chromosome and findings any linkage to the PD performed and high LOD score of 3.6 between two markers on 1p36 chromosomal location(termed PARK 9) have found in that area (Hampshire et al., 2001).

After excluding linkage of this region to other previously identified PARK loci specifically to PARK 6 and PARK 7, loss of function mutation in splice site and also deletion in ATP13A2 gene residing in 1p36 chromosomal region have found (Ramirez et al., 2006). Other mutations in this gene in different kindred were also wrote up later(Di Fonzo et al., 2007).

Following these PARK loci discoveries was a large genetic analysis which used 781 microsatellite markers on 117 patients with age of onset of 65.8 years and 168 unaffected members from 51 Icelandic families and reported linkage of the disease with chromosome 1 (1p32) and designated that locus as PARK 10 (Hicks et al., 2002). However, gene(s) residing in this region responsible for PD manifestations remain to be discovered.

PARK 11 was the next chromosomal location on another PD subtype that discovered in a North American family described before (Pankratz et al., 2002). PD cases in that family shows linkage to PD in 2q36-q37 chromosomal region which designated as PARK 11, but similar to other *Orphan* PD loci the gene and its abnormalities leading to that phenotype is still remains to be discovered (Pankratz et al., 2003b). The study also also described another PD family in which they found linkage to Xq21-q25 chromosomal location commonly called

PARK12(Pankratz et al., 2003a). Some other studies are also maintained linkage of that region to the disease (Hicks et al., 2002, Scott et al., 2001).

The last locus was found between some German PD cases which designated as PARK 13. A candidate gene approach to identify mutation in HTRA2 gene locating in that region resulted in discovery of two G399S and A141S mutations in this gene. More vulnerability to stress-induced cell death is also demonstrated in cells over-expressing G399S mutant HTRA2. In addition, these cells show altered mitochondrial morphology demonstrating direct link between mitochondrial dysfunction and PD neurodegeneration(Strauss et al., 2005).

A Recent a study utilized some of bioinformatics tools to identify and nominate some genes abnormal expression of which may cause PD. They obtained all required data form publicly available three murine studies (Barrett et al., 2001, Stewart et al., 1997, Thuret et al., 2004).

After extracting transcript's sequences they gained 1435 cDNA fragments were that express in midbrain. BLAST search on these cDNA fragments resulted in 579 and 569 corresponding locations on mouse and human genome. Finally comparison of those genes cytogenic locations with previously recorded PARK loci 569 unique locations on human chromosomes 19 of which were located within *orphan* PARK loci have identified (Table 2). In addition more insight in cellular and molecular gene's functions as well as their localization in the cells were obtained by investigation on genes through their associated MGI number. Within these cytogenic locations they could also identified two cDNA fragments inside SNCA, three in NR4A2 and one in *tau* gene which are of nine previously reported genes responsible for PD. By this exploration they suggested some genes in orphan PARK loci mutation(s) of which may could produce PD (Gherbassi et al., 2007).

TABLE 2 : Candidate genes underlying PD disorder suggested by Gherbassi et al. ; a table showing the locations of 19 genes identified in different PARK loci and their related cytogenic locations, human and mouse ID as well as symbol.

No. of fragments aligning the gene	cDNA with	Mouse ID	Human ID	Symbol	Position	PARK Locus
1		NM_146169	XM_376062	KIAA1155	2p13.3	PARK 3
1		NM_008717	NM_014497	ZFML	2p13.2-p13.1	PARK 3
1		NM_183138	XM_371501	MGC22014	2p13.1	PARK 3
3		NM_080555	NM_003713	PPAP2B	1p32	PARK 10
1		AA819910	Estimated	FAF1	1p33	PARK 10
6		NM_009129	NM_003469	SCG2	2q35-q36	PARK 11
3		AK052241	NM_005544	IRS1	2q36	PARK 11
1		NM_152915	NM_139072	DNER	2q37.1	PARK 11
1		NM_0084440	NM_004321	KIF1A	2q37.3	PARK 11
2		NM_024197	NM_004544	NDUFA10	2q37.3	PARK 11
1		NM_025437	NM_001412	EIF1AX	Xp22.13	PARK 12
2		NM_019768	NM_012286	MORF4L2	Xq22	PARK 12
3		NM_011123	NM_000533	PLP1	Xq22	PARK 12
3		NM_013898	NM_004085	TUMM8A	Xq22.1	PARK 12
3		NM_016783	NM_006667	PGRMC1	Xq22-q24	PARK 12
7		NM_030688	Estimated	IL1RAPL2	Xq22.2-q22.3	PARK 12
1		NM_133196	NM_001325	CSTF2	Xq22.1	PARK 12
1		NM_025893	NM_1373798	ZCCHC12	Xq24	PARK 12
2		NM_172782	NM_018698	NXT2	Xq23	PARK 12

Since the available drugs for PD and other neurological disorders act unselectively on the neurons residing in midbrain consisting of both Dopaminergic(SN, VTA and dorsal hypothalamus (DH)) and noradrenergic neurons(locus coeruleus, LC), desired function of a drug for specific disease(like PD) could result in adverse effect in other areas of brain producing new disorder. Of these side effects are paranoia and hallucination which appears in PD patients who received dopamine therapy. This evidence arose some scientist's interests to determine gene expression profile of nuclei in midbrain. Of these

researchers was Grimm who proposed that identification of genes which mainly express in the four nuclei residing in midbrain can provide more insight in disease initiation and development which consequently could lead to discovery of more selectively-act drugs.

In this respect a genome wide screen analyses to identify gene expression profile of these four nuclei on adult female rat performed. After preparing the rat's brain and detection of SN, VTA, DH and LC laser capture microdissection(LCM) utilized and 200 neurons extracted from each group and their RNA isolated. The RNA integrity is well preserved since evaluation of 18S and 28S subunits of rRNAs showed positive result. RNAs were labeled after amplification. Expression of these RNAs also were validated by in situ hybridization before interpretation. Between obtained transcripts those enriched in CA neurons those with 4 fold increased expression in comparison with average expression in whole brain were chosen (1125 transcripts) and clustered according to both where they express and their function.

Analysis and comparison expression differences and similarities between these four nuclei were made and large similarity and overlap between genes express in SN and VTA was found. Their findings demonstrated that SN and VTA have less than 1 % representing 122 genes that express differentially. Various functions of these differentially expressed genes have found containing transcriptional regulator and G protein coupled receptors (Grimm et al., 2004).

Selective degeneration and loss of SN neurons has been shown to cause parkinsonism. Similar observations in this process were made on different animal models. This selective degeneration of SN neurons in comparison with adjacent nuclei, VTA, could results from both increased vulnerability of SN neurons to degeneration and/or increase VTA neurons resistance against degeneration inducers. In searching for clues explaining this differential susceptibility and identify genes participating in those pathway(s) that account for high vulnerability or low defense power of these nuclei a large scale gene expression analysis was performed (Greene et al., 2005). LCM and microarray analysis owing to new advanced technology provide them useful tools for this exploration. 3465 number of cDNA fragments in both SN and VTA were found 144 of which express differentially between SN and VTA. These differentially express genes between

SN and VTA was categorized according to their function and each group was separately investigated. Of these groups was transcription factors binding sites which attracts special interests. More than two third of genes which mainly expressed in SN have putative binding site for NF-E2 and IK-1. This binding sites is decreased to 50 percent in VTA genes. From these and other findings obtained from other groups lead them to the conclusion that vulnerability of SN neurons stem from expression level abnormality of those transcripts participating in energy metabolism.

Another gene expression analysis employed partially same methods of the latter was performed to identify gene expression profile in both SN and VTA. This study used LCM for RNA extraction while demonstrating RNAs integrity by RT-PCR and employed microarray analysis to compare gene expression profiles of both SN and VTA and genes with 2 and 1.5 fold change differences in expression between SN and VTA was extracted(Chung et al., 2005).

MATERIALS AND METHODS :

Database Search:

The genes represented in this study were obtained from six studies, which were aimed at identifying the gene expression profile of midbrain dopaminergic neurons (Thuret et al., 2004, Stewart et al., 1997, Chung et al., 2005, Greene et al., 2005, Grimm et al., 2004). The list of genes was prepared using the raw data and supplementary material from the three latter studies. Since two of the studies were performed on rats and the other on mice, in order to combine the lists of genes, we found the analogous genes in the mouse genome by searching the rat genes names in nucleotide database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>) of the NCBI webpage (<http://www.ncbi.nlm.nih.gov/sites/entrez/>) and doing a BLAST (Basic Local alignment Search Tool, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) search, using the mRNA sequence for each gene.

Data validation:

In order to confirm the expression of candidate genes, in SNpc and VTA, I used the mouse brain expression database from Allen Brain Institute (<http://www.brain-map.org/welcome.do>) (Lein et al., 2007)

This page provide useful tools for detection of expression of huge number of genes in various mice brain regions. After fixing and slicing mice brains in different planes and using in situ hybridization staining method against mRNA of gene of interest high quality images obtained from these sections and arranged one after another.

By searching gene name or symbol in this web page at least one series(coronal or sagittal series) of this stained images will appear. Pictures in

different planes either from same or different genes could be compared by adding their series into thumbnail view.

All genes of interest were searched separately in this web site and coronal and/or sagittal views of brains cuts compared to specific gene as positive control which mainly expresses both SN and VTA in high level. Of these are vesicular monoamine transporter 2 (VMAT-2) and Tyrosin hydroxylase (TH). I had chosen TH as a positive control since it express in midbrain exclusively in both SN and VTA. Through this method I could confirm expression of extracted genes in SN or VTA.

Gene locations on human genome and mapping into the PARK loci

To identify locations of the genes, analogous to the mouse genes, on the human genome, the Map Viewer tool of NCBI was used (<http://www.ncbi.nlm.nih.gov/mapview/>).

Then, the recorded chromosomal locations were mapped into the previously described PARK loci at (www.pdgene.org) and a list of genes, which fell into the recorded PARK loci, was constructed for further analysis.

RESULTS :

Transcripts collection and processing:

Several gene abnormalities in 8 out of 13 PARK loci have been identified. The remaining PARK loci, called *orphan PD loci*, contain yet-to-be-discovered genes. These PARK loci span from 7 Mb to 40 Mb on different chromosomes and contain hundreds of genes. Identification of new PD genes requires proper criteria to narrow down the large number in a rational manner.

To date, six screens have studied the gene expression pattern in rodent mesDA neurons (Thuret et al., 2004, Stewart et al., 1997, Chung et al., 2005, Greene et al., 2005, Grimm et al., 2004) with special attention to SN and VTA. Recently, a survey have linked the data from three of the studies, Barrett et al., Stewart et al. and Thuret et al., to the orphan PD loci. They have identified 19 genes within orphan OMIM PD loci, with three known PD genes, being among them (Barrett et al., 2001, Stewart et al., 1997, Thuret et al., 2004). In this study I have employed a novel approach to map the transcripts obtained from the other three studies, two of which explored gene expression in rats and another one on mice, to nominate some other genes in PARK loci which may be responsible for PD (Grimm et al., 2004, Greene et al., 2005, Chung et al., 2005).

I extracted genes which are expressed in SN, regardless of their expression in VTA from three above mentioned studies and put them all in a separate tables. Respectively 95, 100 and 51 genes with total number of 246 were acquired that have been shown that significantly have more expression in SN in comparison to VTA. Other genes that were expressed equally were also added to the table on which following steps of the research have done (Table 3).

Study	Total number of genes	SN and VTA	Located in PARK loci
Chung et al.	383	95	11
Greene et al.	137	51	2
Grimm et al.	412	100	7
Total	932	246	20

TABLE 3: The total number of genes within each study and the number of genes, located in PARK loci for each study; three studies were employed for detection of nominated genes for PD.

Unification of genes based on mice genome:

Since gene information including gene accession number, symbol and even their names differ between organisms, and two of three selected studies performed on rats and one on mice, the findings were heterogeneous and I should first make my data homogenous based on mice genome. I unified my data based on mice genome, but not based on rats, due to presence of large similarities between human and mouse genome information that makes my future attempts easier to accomplish.

In order to do BLAST search, required for making information homogenous, I first need to have nucleotide sequence of gene of interest. To this end I searched the gene's accession numbers in nucleotide data base on NCBI. Through this method several reports as well as complete information of that gene including its nucleotide sequence at the bottom of the result page will appear.

Each nucleotide sequence was temporarily recorded and employed as a query for nucleotide-nucleotide BLAST search. Through this search tool I could gained multiple reports of alignments from the query sequence in different experimental animals range from yeast to mice with their related information. Best-suited report is that with highest score, lowest e value and maximum number of hits. The best report was selected and their required information (including accession and serial number as well as gene symbol) to fulfill the gaps in that table were extracted.

These methods let me construction of a table containing plenty of genes, which express in SNpc neurons. Then all recorded genes set up and repetitive ones were revealed out.

Validation of Results:

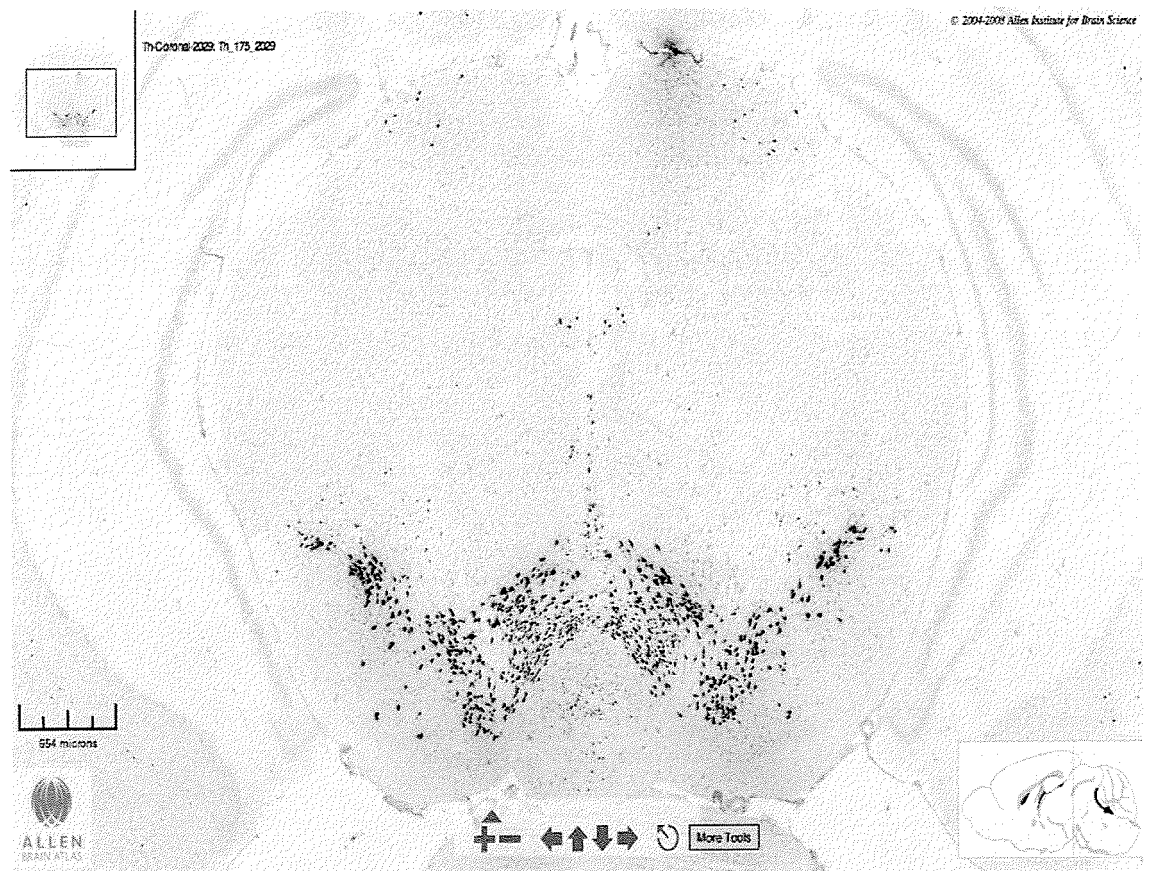
Greene and his coworkers confirmed gene expression in those nuclei by both quantitative real time PCR and also by reviewing published literatures. The two other studies are also validate their findings by in situ hybridization and real time PCR respectively. Thus all presented genes in that table have been shown primarily by their authors to express in SN and VTA neurons. However although all derived genes from these studies were validated previously, as an independent, credential and self-explanatory study I should also confirmed expression of obtained and processed genes in SN neurons.

To this end I should first detect expression of gene of interest in mouse brain. I could stain prepared mice brains and see whether these genes express in specific brain region(SN or VTA) or not as had done by latter studies. However I used a web page prepared by Allen Brain atlas within which pictures from mice brain's cut that stained with in situ hybridization against multiple genes are placed. This separate staining show expression pattern of special gene in different brain regions. In this method special probe to explore specific mRNA are employed. Probes then stained and thin sections(coronal and / or sagittal) cuts derived from prepared mice brains. High quality pictures taken from this in situ hybridized sections and are added as arranged series to this web page. Due to some limitation in producing 3D images they show the cuts as plane pictures in sagittal and coronal cuts like those obtain in CT-Scan or MR imaging.

Although these cuts are present in these pages, reliable indicators (as control) such as proteins which mainly express in these two nuclei, for accurate detection of SN and VTA nuclei is needed. The brain atlas web page provide another useful tool by which two genes could be compared simultaneously. Thus by choosing suitable protein indicator for SN and VTA anatomical areas and

comparing it with gene of interest I could confirm expression of the genes in mentioned areas.

Several genes have been shown to express mainly in SN and VTA neurons and could be choose as suitable positive control. Of these are vesicular monoamine transporter 2 (VMAT-2) and Tyrosin hydroxylase (TH). I had chosen TH as a positive control which could be good indicator for both SN and VTA regions. One cut from those demonstrating TH expression, indicating SN and VTA borderlines, were selected and compared with the same cut representing expression of gene of interest. All obtained genes expression were individually confirmed by this way. By this method I could validate expression of obtained genes from above three mentioned studies in SN and VTA neuronal cells .



a)



Figure 10: In situ hybridization, performed on a coronal section of mouse brain and its anatomical locations. a) Coronal picture of a mouse brain showing TH expression in two nuclei of VTA, medial trapezoid structures close to midline, and SN which is located lateral to VTA, separated by a less dense area. b) One coronal cut of cerebral stained hemisphere of a mouse and one schematically shown brain nuclei in front of it for comparison.

Cytogenic locations:

After confirming expression of selected genes in SN and VTA regions of mice brain detection of location of these genes seems necessary. Another tool present in NCBI web page is Map Viewer by which we could search for chromosomal locations of each genes in various animals even homo sapiens. Human and mouse genome show to have so much similarities in respect to their genome. Their genes almost have same name, symbols and chromosomal location and this was the reason for the unification of data based on mice genome in previous steps.

I searched all the obtained genes in Map Viewer page to detect their chromosomal locations in homo sapiens. Cytogenic locations of genes of almost all murine could be found in homo sapiens chromosome with same name and symbol which were extracted and recorded in the table. However there were some genes which could not found as gene in homo sapiens genome. For detection of chromosomal location of these scarce genes I searched their accession number in Nucleotide data base of NCBI and record other aliases for the gene and use those for detection of gene location in homo sapiens. Through this method I could found all gene's chromosomal locations. The cytogenic locations of all genes were recorded to the table and prepared for the final step which is comparing these locations with previously recorded PARK loci.

Correlating genes location with previously recorded PARK loci

For last step I should check the genes chromosomal locations with previously described PARK loci. Another web page instructed by Harvard university put valuable information about AD and PD, two most common neurodegenerative disorder. In this web page several information about PARK loci including PARK loci schematic diagram as their distances (Figure 11).

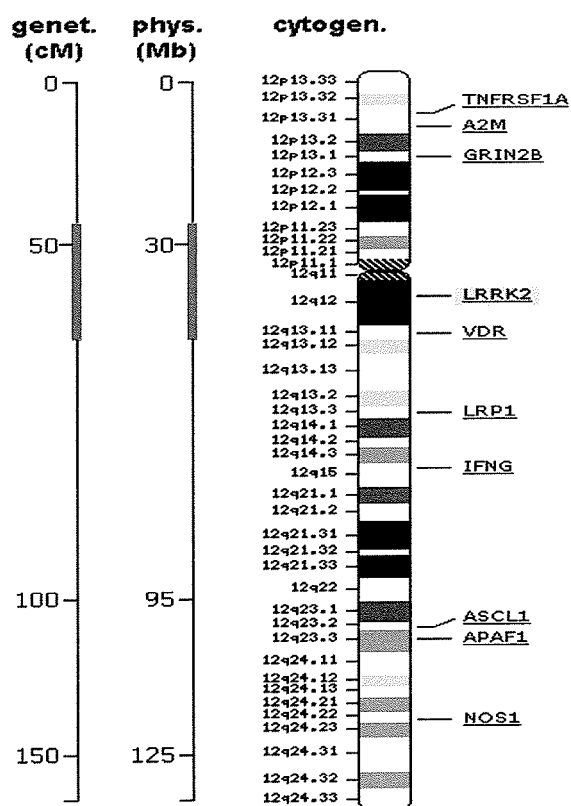


Figure 11. Schematic diagram of a PARK locus presented in PDGENE web page.
 Chromosomal location of PARK 8 occupying centromeric region on chromosome 12 (distance : 12p11-23-q13.11)

All genes from this have been compared with the PARK loci and those within these locations were recorded (appendix). By these approaches I could find 20 genes in different PARK loci which could be nominated for further investigations (Table 4).

Gene name	Gene accession number	Gene accession number	Screen	position	Locus
Tetraspanin 6		Tspan6	Chung	Xq22.1	PARK 12
Huntingtin interacting protein 2	NM_016786	HIP2	Chung	4p14	PARK 5
Ribosomal protein L11	NM_025919	RPL11	Chung	1p36.1-p35	PARK 6 & 7
ribosomal protein L36a	NM_019865	RPL36A	Chung	Xq22.1	PARK 12
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	NM_053123	SMARCA1	Chung	Xq25	PARK 12
MARCKS-like 1	NM_010807	MARCKSL1	Chung	1p35.1	PARK 6
GTPase activating protein 24	NM_029270	ARHGAP24	Chung	4q21.23-4q21.3	PARK 4
F-box only protein 2	NM_176848	FBXO2	Chung	1p36.22	PARK 7
I-complex-associated-testis-expressed 1-like cyclin I	NM_017367	DYNLT3	Chung	Xp21	PARK 12
CCNI	NM_017367	CCNI	Chung	4q21.1	PARK 4
solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5	NM_007451	SLC25A5	Chung	Xq24-q26	PARK 12
pyruvate dehydrogenase E1 alpha 1	NM_008810	PDHA1	Greene	Xp22.12	PARK 12
ubiquitin carboxy-terminal hydrolase L1	NM_011670	UCHL1	Greene	4p14	PARK 5
G protein-coupled receptor, family C, group 5, member A	NM_181444	GPRC5A	Grimm	12p13.1	PARK 8
Protein tyrosine phosphatase, receptor type, U	NM_011214	PTPRU	Grimm	1p35.3	PARK 6
kelch-like 13 (Drosophila)	NM_026167	KLHL13	Grimm	Xq24	PARK 12
dehydrogenase/reductase (SDR family) member 3	NM_011303	DHRS3	Grimm	1p36.22-1p36.21	PARK 7
NEL-like 2 (chicken)	NM_016743	NELL2	Grimm	12q13.11-q13.12	PARK 8
Serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_009254	SERPINB6	Grimm	6q25	PARK 2
peripherin 1	NM_013639	PRPH	Grimm	12q12-q13	PARK 8

TABLE 4: Nominated genes identified by this study. 20 genes were identified on human chromosome which are located on previously detected PARK loci, 12 of which being within orphan PD loci.

DISCUSSION AND FUTURE WORKS :

The causes for the appearance of the pathological hallmark of PD, loss of mesencephalic dopaminergic neurons in the substantia nigra pars compacta are unknown. The mechanism of formation of protein aggregates or LB's, the role of energy metabolism and mitochondrial dysfunction are also not fully understood and subject to extensive investigation. Therefore, no drug has been discovered to halt neuronal degeneration and available medications merely alleviate the disease symptoms. Further understanding of pathways, leading to familial and sporadic forms of PD may lead into development or identification of therapeutic reagents, which can overcome this problem and in addition to halting the disease progression, lead to durable cure.

Genetic linkage studies employ bioinformatics tools to detect susceptibility genes, alterations in which could results in PD. Based on current understanding, obtained from linkage studies, thirteen loci on different human chromosomes have been shown connection to PD, which are called PARK loci. Although some abnormalities in genes in these loci have been shown, there are multiple yet-to-be-discovered genes in other PARK (orphan) loci, which require further investigation.

Recently a study made use of this approach to identify genes that may underlies PD pathogenesis. They employed findings obtained from three murine linkage studies and used publicly available genetic databases to link them with orphan PARK loci. By analyzing transcripts of dopaminergic neurons in midbrain, obtained from three murine studies and merging these findings with recently mapped human chromosome, 19 genes were identified as being located in the orphan PD loci and candidates for PD genes. (Gherbassi et al., 2007)

In this work, I employed similar methods to identify candidate genes underlying PD. I collect my transcripts from three additional screens which were performed to identify gene expression profile of midbrain neurons in rats and mice. Then I made the data homogenous based on mouse genome and checked

the expression of obtained transcripts within SN and/or VTA. Locating mice gene's position on human chromosome was the next task after which I compared the gene locations with previously described PARK loci. This method led to identification of 20 genes within PARK loci. This results showed, for the first time, that 19 of the genes, expressed within dopaminergic neurons of midbrain, lie within PD loci, 9 of which are located in orphan PD loci (Figure 12).

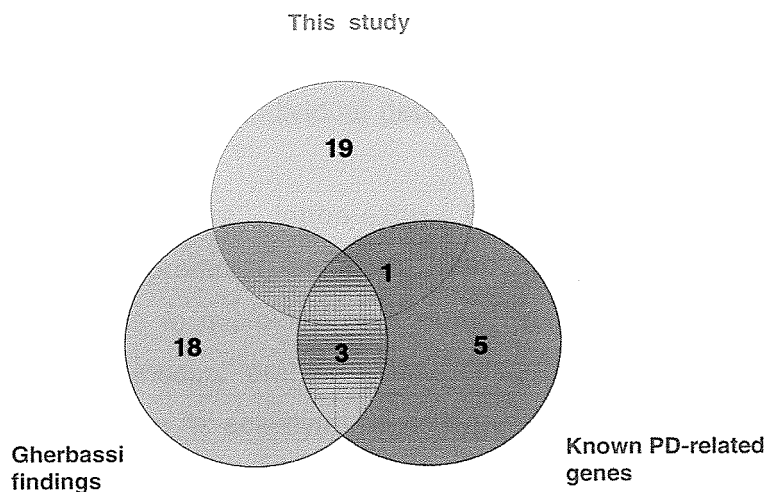


Figure 12. Venadiagram shows number of genes find by that study .

In this study (green circle) I could identify twenty genes one of (UCHL1) which have been previously shown to have linked to PD (brown circle).

One of the 20 candidate genes, UCHL1, was previously identified as a responsible gene for PD. Presence of this gene among the results of this study strongly validates the methods used, since this gene was identified by an objective method from hundreds of other expressed transcripts within this region. Double checking the expression of all 20 genes in Allen Brain Atlas show expression of the all genes predominantly or exclusively in the SNpc. This is another indication of the validity of the criteria of expression within midbrain dopaminergic neurons for narrowing down the number of candidate genes as the starting point of the study, since the dopaminergic neurons in SNpc are more vulnerable to degeneration during the course of Parkinson's disease.

possibility of involvement of developmental or postnatal molecular cascades in appearance of PD.

Another important factor playing a role in incidence of PD is sex. Many animals have shown to be sexually dimorphic in variety of features. The differences are not only in structural characteristics like body size, brain size and weight, skeletal system, but also other features like gene expression as well as behavioral differences like reproductive, aggression and domination behavior is also recorded as sexually dimorphic features(Reinius et al., 2008). These differences in brain regions may result from hormonal differences. However, recently multiple studies have been shown that sex-linked as well as autosomally located genes are expressed differentially between genders(Dewing et al., 2003). Studies show that some brain regions specifically affected by hormones however some other areas needs both hormones and differential genes expression in sexes to form normally according to their gender. In addition some areas of the brains shows unresponsiveness to hormones indicating their dependence solely on differential chromosomal expression(Davies et al., 2006).

The incidence of PD, as mentioned earlier in introduction, is not equally distributed between genders and several studies have shown that males are more vulnerable (approx. 2 fold) to the disease than females. A recent study performed on SN neurons of human has shown more than 2000 genes expressed differentially between human genders(Cantuti-Castelvetri et al., 2007). Of this number, some were sex-linked however in general the majority of genes were not sex-linked.

In addition they compared and analyzed gene expression differences between PD patients and healthy controls of both genders and identified the differentially expressed genes. Interestingly, five of the genes(HIP2, SMRCA1, FBXO2, SLC25A5 and KLHL13), identified in this study are among the differentially expressed between sexes. I found that all detected genes are up-regulated in males and/or down regulated in females, suggesting direct relation between expression and vulnerability to PD. In addition, I found one gene (GPRC5A) that was down-regulated in female PD patients in comparison to female controls (Cantuti-Castelvetri et al., 2007).

Comparison between only	Total number in Cantuti-Castelvetri study	nominated genes in this study	Situation
Male / female irrespective to disease manifestation	2120	5	Down-regulated in females or up regulated in males
Control / PD irrespective to gender	145	0	
Female PD / female control	~288	1	Down regulated in PD female or up regulated in female control
Male PD / male controls	~292	0	

TABLE 5 . Results of a study by Cantuti-Castelvetri et al. and compare it with nominated genes in this study . This comparison shows that 5 out of 21 candidate genes differentially express between males and females and 1 gene out of 21 were down regulated PD afflicted females and/or up regulated control females.

In this study 20 genes were identified from thousands of genes present in PARK loci, 12 of which are located in orphan PD loci. These genes could be further evaluated by clinical studies of familial as well as sporadic PD cases, to see whether any of the candidates is responsible or plays a role in progression of the disease. These genes also may play a role in survival and maintenance of the dopaminergic neurons, therefore, their functions, with regard to mesDA neurons, can be evaluated in transgenic as well as neurotoxin-based animal models of PD.

CONCLUSION :

Parkinson's disease is the second most popular neurodegenerative disorder affecting about 1 % of individuals more than 65. In addition to idiopathic, hereditary form of this disorder also exist. Several familial aggregation studies provide well documented cases of hereditary PD cases. Further investigations on these individuals by linkage studies reveal different chromosomal locations that link to the disease. Until now thirteen chromosomal locations have been shown to link to PD. Attempts to identify responsible genes in these locations results in detection of some genes in some these loci however responsible genes in some other locations remains to be discovered (commonly called orphan PD loci).

Since degeneration of neurons residing in substantia nigra (SN) but not in other areas of the brain even the closest nucleolus, ventral tegmentum area (VTA), causes PD, I employ this unique feature of PD to nominate some genes in orphan PD loci mutation of which might result in PD pathogenesis. By extracting genes that mainly express in SN from three murine studies and detecting their location and correlating them with human chromosomal locations I could candidate 20 genes in PD loci 9 of which are located in orphan PD loci.

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APPENDICES:

Gene Name	Gene Symbol	Screen	Accession Number	Chromosome location	cytogenic location	PD loci correlation
aldo-keto reductase family 1, member B3	Akr1b3	Grimm	NM_009658	non	AKR1B1(aldose reductase) = 7q35	no
cholinergic receptor, nicotinic, alpha polypeptide 4	Chrna4	Grimm	NM_015730	20q13.33		no
glial cell line derived neurotrophic factor family receptor alpha 1	Gfra1	Grimm	NM_010279	non	GFRA1 = 10q25.3	no
tyrosine hydroxylase	Th	Grimm	NM_009377	11p15.5		no
tyrosine hydroxylase	Th	Grimm	NM_009377	11p15.5		no
peripherin 1	Prph1	Grimm	NM_013639	non	PRPH = 12q12-q13	Inside PARK 8
dopa decarboxylase	Ddc	Grimm	NM_016672	7p12.2		no
pterin 4 alpha carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 1	Pcbd1	Grimm	NM_025273	non	PCDB1 = 10q22	no
cell growth regulator with EF hand domain 1	Cgref1	Grimm	XM_181420	2p23.3		near PARK 3
dopamine receptor D1 interacting protein	Drd1ip	Grimm	NM_026769	10q26.3		no
protein arginine N-methyltransferase 2	Prmt2	Grimm	NM_133182	21q22.3		no
TSPY-like 2	Tspyl2	Grimm	NM_029836	Xp11.22		no
aldehyde dehydrogenase family 1, subfamily A1	Aldh1a1	Grimm	NM_013467	non	ALDH1A1 = 9q21.13	no
Down syndrome critical region gene 1-like 1	Dscr11i	Grimm	NM_030598	6p12.3		no
RNA binding motif, single stranded interacting protein	Rbms3	Grimm	NM_178660	3p24.1		no
protein kinase C, alpha	Prkca	Grimm	NM_011101	17q24.1-17q24.2		no
RIKEN cDNA 2010011I20 gene	2010011I20Rik	Grimm	NM_025912	non		
Ly6/Plaur domain containing 1	Lypd1	Grimm	NM_145100	2q21.2		near PARK 11
solute carrier family 39 (zinc transporter), member 4	Slc39a4	Grimm	NM_028064	8q24.3		no
serine (or cysteine) peptidase inhibitor, clade B, member 6a	Serpib6a	Grimm	NM_009254	non	SERPINB6 = 6q25	Inside PARK 2
NEL-like 2 (chicken)	Nell2	Grimm	NM_016743	non	NELL2 = 12q13.11-q13.12	Inside PARK 8
potassium voltage-gated channel, Shal-related family, member 3	Kcnd3	Grimm	NM_001039347	non	KCND3 = 1p13.3	no
protein kinase C, epsilon	Prkce	Grimm	NM_011104	2p21		no
leucine rich repeat containing 3B	Lrrc3b	Grimm	NM_146052	3p24.1		no
serine/threonine kinase 39, STE20/SPS1 homolog (yeast)	Stk39	Grimm	NM_016866	non	STK39 = 2q24.3	Near PARK 11
RIKEN cDNA A030009H04 gene	A030009H04Rik	Grimm	NC_000077.5	non		
DNA segment, Chr 14, ERATO Doi 171, expressed	D14Ert171e	Grimm	NM_177814	non	Record were replaced CAST = 5q15	no
glucan (1,4-alpha-), branching enzyme 1	Gbe1	Grimm	NM_028803	3p12.3-3p12.2		no
dehydrogenase/reductase (SDR family) member 3	Dhrs3	Grimm	NM_011303	1p36.22-1p36.21		inside PARK 7, PARK 9 & near PARK 6
kelch-like 13 (Drosophila)	Klhl13	Grimm	NM_026167	Xq24		Inside PARK 12

aurora kinase A (or serine/threonine protein kinase 15)	Aurka	Grimm	NM_011497	20q13.2-q13.3		no
LIM domain only 4	Lmo4	Grimm	NM_010723	1p22.3		no
phospholipase C, beta 1	Plcb1	Grimm	NM_019677	20p12.3-20p12.2		no
cytochrome b-561	Cyb561	Grimm	NM_007805	17q23.3		no
CUG triplet repeat, RNA binding protein 2	Cugbp2	Grimm	NM_010160	10p14		no
latrophilin 2	Lphn2	Grimm	XM_888040	1p31.1		Near PARK 10
protein tyrosine phosphatase, receptor type, U	Ptpru	Grimm	NM_011214	1p35.3		Inside PARK 6 & Near PARK 9 & 7
cDNA sequence BC065085	BC065085	Grimm		non		
solute carrier organic anion transporter family, member 4a1	Slco4a1	Grimm	NM_148933	20q13.33		no
cadherin 13	Cdh13	Grimm	NM_019707	16q23.2-16q24.1		no
serine (or cysteine) peptidase inhibitor, clade E, member 2	Serpine2	Grimm	NM_009255	non	SERPINE2 = 2q33-q35	Near PARK 11
pleckstrin homology domain containing, family A member 7	Plekha7	Grimm		11p15.1		no
angiomin-like 1	Amotl1	Grimm	XM_919481	non	AMOTEL1 = 11q14.3	no
pre B-cell leukemia transcription factor 1	Pbx1	Grimm	NM_008783	non	PBX = 1q23	no
ets variant gene 5	Etv5	Grimm	NM_023794	3q27.2		no
RIKEN cDNA D330050I23 gene	D330050I23Rik	Grimm		non		
Purkinje cell protein 4-like 1	Pcp4l1	Grimm	XM_484933	non	PCP4L1 = 1q23.3	no
endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	Edg2	Grimm	NM_010336	9q31.3		no
teashirt zinc finger family member 1	Tshz1	Grimm	XM_888252	non	TSHZ1 = 18q22.3	no
glutamate receptor interacting protein 2	Grip2	Grimm	XM_001000130	3p25.1		no
par-3 (partitioning defective 3) homolog (C. elegans)	Pard3	Grimm	NM_001013580	10p11.22-10p11.21		no
Ras association (RalGDS/AF-6) domain family 6	Rassf6	Grimm	NM_028478	non	RASSF6 = 4q13.3	no
eyes absent 2 homolog (Drosophila)	Eya2	Grimm	NM_010165	20q13.12		no
RIKEN cDNA 2700045P11 gene	2700045P11Rik	Grimm		non		
AT motif binding factor 1	Atbf1	Grimm	NM_007496	non	ZFX3 = 16q22.3-q23.1 , PIAS3 = 1q21	no
solute carrier family 31, member 1	Slc31a1	Grimm	NM_175090	9q32		no
rhodopsin	Rho	Grimm	NM_145383	3q21.3		no
glycoprotein (transmembrane) nmb	Gpnmb	Grimm	NM_053110	non	GPNUMB = 7q15	no
Ca2+-dependent activator protein for secretion 2	Cadps2	Grimm	NM_153163	non	CADPS2 = 7q31.3	no
fatty acid desaturase 3	Fads3	Grimm	NM_021890	11q12.2-11q12.3		no
calcium-sensing receptor	Casr	Grimm	NM_013803	3q13.33-3q21.1		no
transmembrane inner ear	Tmie	Grimm	NM_146260	3p21.31		no
glutathione peroxidase 3	Gpx3	Grimm	NM_008161	non	GPX3 = 5q23	no
RIKEN cDNA A930038C07 gene	A930038C07Rik	Grimm	NM_172399	non		
fibronectin type III domain containing 3B	Fndc3b	Grimm		3q26.31		no
protein phosphatase 1, regulatory (inhibitor) subunit	Ppp1r14b	Grimm	NM_008889	non	PPP1R14B = 11q13	no

14B						
myosin Vb	Myo5b	Grimm	NM_201600	18q21.1		no
phosphofructokinase, liver, B-type	Pfkf	Grimm	NM_008826	non	PFKL = 21q22.3	no
thyroid transcription factor 1	Titf1	Grimm	NM_009385	14q13.3		no
claudin 1	Cldn1	Grimm	NM_016674	non	CLDN1 = 3q28-q29	no
guanine nucleotide binding protein (G protein), gamma 11	Gng11	Grimm	NM_025331	non	GNG11 = 7q21.3	no
scrapie responsive gene 1	Scrg1	Grimm	NM_009136	4q34.1		no
X-box binding protein 1	Xbp1	Grimm	NM_013842	22q12.1		no
G protein-coupled receptor, family C, group 5, member A	Gprc5a	Grimm	NM_181444	12p13.1		Inside PARK 8
phosphoglucomutase 2-like 1	Pgm2l1	Grimm		11q13.4		no
regulator of G-protein signaling 2	Rgs2	Grimm		non	RGS2 = 1q31	no
cytochrome P450, family 51	Cyp51	Greene	NM_020010	non	CYP51A1 = 7q21.2-q21.3	no
ubiquitin carboxy-terminal hydrolase L1	Uchl1	Greene	NM_011670	non	UCHL1 = 4p14	Inside PARK 5
brain derived neurotrophic factor	Bdnf	Greene	NM_001048139	non	BDNF = 11p13	no
pyruvate dehydrogenase E1 alpha 1	Pdha1	Greene	NM_008810	Xp22.12		inside PARK 12
phospholipase C, beta 4	PLCB4	Greene	NM_013829	20p12.3-20p12.2		no
lysophospholipase 1	Lypla1	Greene	NM_008866	8q11.23		no
solute carrier family 24 (sodium/potassium/calcium exchanger), member 2	Slc24a2	Greene		9p22.1-9p21.3		no
ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	Atp2a2	Greene	NM_009722	12q24.11		no
calpain, small subunit 1	Capns1	Greene	NM_009795	19q13.12		no
inositol 1,4,5-triphosphate receptor 1	Itpr1	Greene	NM_010585	3p26.2		no
cysteine rich protein 2	Crip2	Greene	NM_024223	non	CRP2 = 14q32.3	no
dynein cytoplasmic 1 intermediate chain 1	Dync1i1	Greene	NM_010063	non	DYNC1I1 = 7q21.3-q22.1	no
ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	UQCRCF1	Greene	NM_025710	19q12		no
ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	Atp5a1	Greene	NM_007505	18q21.1		no
glutamate oxaloacetate transaminase 2, mitochondrial	Got2	Greene	NM_010325	non	GOT2 = 16q21	no
pyruvate kinase, muscle	Pkm2	Greene	NM_011099	non	PKM2 = 15q22	no
creatine kinase, brain	Ckb	Greene	NM_021273	non	CKB = 14q32	no
adenylate kinase 1	Ak1	Greene	NM_021515	non	AK1 = 9q34.1	no
guanine nucleotide binding protein, alpha o	Gnao1	Greene	NM_010308	16q13		no
enolase 3, beta muscle	Eno3	Greene	NM_007933	17p13.2		no
dopamine receptor 2	Drd2	Greene	NM_010077	non	DRD2 = 11q23	no
calcium channel, voltage-dependent, T type, alpha 1G subunit	Cacna1g	Greene	NM_009783	17q21.33		no
protein kinase C, beta 1	Prkcb1	Greene	NM_008855	16p12.1		no
protein kinase, cAMP dependent regulatory, type II beta	Prkar2b	Greene	NM_011158	non	PKRKAR2B = 7q22.3	no
proprotein convertase subtilisin/kexin type 2	Pcsk2	Greene	NM_008792	20p12.1		no
neurtin 1	Nrn1	Greene	NM_153529	6p25.1		no
monoamine oxidase A	Maoa	Greene	NM_173740	non	MAOA = Xp11.3	no
GTP cyclohydrolase 1	Gch1	Greene	NM_008102	14q22.2-14q22.3		no
solute carrier family 7 (cationic amino acid transporter, y+ system),	Slc7a3	Greene	NM_007515	non	SLC7A3 = Xq13.1	Near PARK 12

member 3						
cathepsin D	Ctsd	Greene	NM_009983	non	CTSD = 11p15.5	no
vesicle-associated membrane protein 2	Vamp2	Greene	NM_009497	17p13.1		no
transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	Tle1	Greene		9q21.31-9q21.32		no
growth factor receptor-bound protein 10	Grb10	Greene		7p12.3-7p12.1		no
target of myb1-like 2 (chicken)	Tom1l2	Greene		17p11.2		no
RIKEN cDNA A230083H22 gene	A230083H22Rik	Greene		non	KIAA0367 = 9q21.13 ?	no
calbindin-28K	Calb1	Chung	NM_009788	non	CALB1 = 8q21.3-q22.1	no
guanosine diphosphate (GDP) dissociation inhibitor 2	Gdi2	Chung	NM_008112	non	GDI2 = 10p15	no
longevity assurance homolog 4	Lass4	Chung	NM_026058	19p13.2		no
microtubule-associated protein 1 light chain 3 alpha	Map1lc3a	Chung	NM_025735	20q11.22		no
nuclear receptor interacting protein 3	Nrip3	Chung	NM_020610	11p15.4		no
CD24a antigen	Cd24a	Chung	NM_009846	non	CD24 = 6q21	Near PARK 2
RAB3C, member RAS oncogene family	Rab3c	Chung	NM_023852	5q11.2		no
acyl-CoA synthetase long-chain family member 6	Acsf6	Chung	NM_001033597	5q31.1		no
myosin Va	Myo5a	Chung	NM_010864	15q21.2		no
RAR-related orphan receptor alpha	Rora	Chung		15q22.2		no
syndecan 2	Sdc2	Chung	NM_008304	8q22.1		no
fibroblast growth factor 1	Fgf1	Chung	NM_010197	non	FGF1 = 5q31	no
poliovirus receptor-related 3	Pvrl3	Chung	NM_021495	3q13.13		no
glutamate receptor, ionotropic, NMDA2C (epsilon 3)	Grin2c	Chung	NM_010350	non	GRIN2C = 17q25	no
zinc finger, DHHC domain containing 2	Zdhhc2	Chung	NM_178395	8p21.3-p22		no
cytochrome P450, family 4, subfamily v, polypeptide 3	Cyp4v3	Chung	NM_133969	non	non	
special AT-rich sequence binding protein 1	Satb1	Chung	NM_009122	3p24.3		no
acetyl-Coenzyme A dehydrogenase, long-chain	Acadl	Chung	NM_007381	non	ACADL = 2q34-q35	Near PARK 11
solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5	Slc25a5	Chung	NM_007451	non	SLC25A5 = Xq24-q26	Inside PARK 12
vav 3 oncogene	Vav3	Chung	NM_020505	1p13.3		no
calcium channel, voltage dependent, alpha2/delta subunit 3	Cacna2d3	Chung	NM_009785	non	CACNA2D3 = 3p21.1-3p14.3	no
synuclein, gamma	Sncg	Chung	NM_011430	10q23.2-q23.3		no
oxysterol binding protein-like 11	Osbpl11	Chung	NM_176840	3q21.2		no
limb expression 1 homolog (chicken)	Lix1	Chung	NM_025681	non	LIX1 = 5q15	no
annexin A1	Anxa1	Chung		9q12-q21.2		no
Ca ²⁺ -dependent activator protein for secretion	Cadps	Chung	NM_001042617	non	CADPS = 3p14.2	no
RAS-like, family 11, member B	Rasl11b	Chung	NM_026878	4q12		Near PARK 4
polymerase (DNA directed), beta	Polb	Chung		non	POLB = 8p11.21	no
cyclin I	Ccni	Chung	NM_017367	non	CCNI = 4q21.1	Inside PARK 4
RNA terminal phosphate cyclase domain 1	Rtcd1	Chung	NM_025517	1p21.2		no
lysosomal-associated protein	Laptm4b	Chung	NM_033521	non	LAPTM4B =	no

transmembrane 4B					8q22.1	
cadherin 8	Cdh8	Chung	NM_001039154	16q21		no
ganglioside-induced differentiation-associated-protein 1	Gdap1	Chung	NM_010267	non	GDAP1 = 8q21.11	no
adenosine kinase	Adk	Chung	NM_134079	10q22.2		no
t-complex-associated-testis-expressed 1-like	Tcte1l	Chung		non	DYNLT3 = Xp21	Inside PARK 12
aspartyl aminopeptidase	Dnpep	Chung	NM_016878	2q35		Near PARK 11
regulator of G-protein signaling 8	Rgs8	Chung	NM_026380	non	RGS 8 = 1q25	no
pre B-cell leukemia transcription factor 3	Pbx3	Chung	NM_016768	non	PBX 3 = 9q33-q34	no
trophoblast glycoprotein	Tpbp	Chung	NM_011627	6q14.1		Near PARK 2
CDP-diacylglycerol synthase (phosphatidate cytidyltransferase) 2	Cds2	Chung	NM_138651	non	CDS2 = 20p13	no
myocyte enhancer factor 2A	Mef2a	Chung	NM_001033713	15q26.3		no
mitogen activated protein kinase 9	Mapk9	Chung	NM_016961	non	MAPK9 = 5q35	no
F-box only protein 2	Fbxo2	Chung	NM_176848	1p36.22		inside PARK 7 & near PARK 6 and PARK 9
macrophage migration inhibitory factor	Mif	Chung	NM_010798	non	MIF = 22q11.23	no
NADH dehydrogenase (ubiquinone) Fe-S protein 8	Ndufs8	Chung	NM_144870	non	NDUFS8 = 11q13	no
ELOVL family member 6, elongation of long chain fatty acids (yeast)	Elovl6	Chung	NM_130450	4q25		Near PARK 4
CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	Cd47	Chung	NM_010581	3q13.12		no
citrate synthase	Cs	Chung	NM_026444	non	CS = 12q13.2-q13.3	Near PARK 8
phosphatidylinositol transfer protein alpha	Pitpna	Chung	NM_008850	17p13.3		no
homer homolog 2 (Drosophila)	Homer2	Chung	NM_011983	15q25.2		no
RASD family, member 2	Rasd2	Chung	XM_204287	22q12.3		no
thioredoxin domain containing 14	Txndc14	Chung	NM_025868	11q12.1		no
3-oxoacid CoA transferase 1	Oxct1	Chung	NM_024188	5p13.1		no
translocase of outer mitochondrial membrane 20 homolog (yeast)	Tomm20	Chung	NM_024214	1q42.3		no
N-ethylmaleimide sensitive fusion protein attachment protein beta	Napb	Chung	NM_019632	non	NAPB = 20p12.3-p11.21	no
amyloid beta (A4) precursor protein-binding, family A, member 2	Apba2	Chung	NM_007461	non	APBA2 = 15q11-q12	no
NIMA (never in mitosis gene a)-related expressed kinase 7	Nek7	Chung	NM_021605	1q31.3		no
vacuolar protein sorting 35	Vps35	Chung	NM_022997	16q11.2		no
RAB6, member RAS oncogene family	Rab6	Chung	NM_024287	non	RAB6A = 11q13.3	no
very low density lipoprotein receptor	Vldlr	Chung	NM_013703	non	VLDLR = 9p24	no
GTPase activating protein 24	Arhgap24	Chung	NM_029270	4q21.23-4q21.3		Inside PARK 4
visinin-like 1	Vsnl1	Chung	NM_012038	2p24.2		no
protein tyrosine phosphatase, non-receptor type 5	Plpn5	Chung	NM_013643	11p15.1		no
prohibitin 2	Phb2	Chung	NM_007531	12p13.31		Near PARK 8
NADH dehydrogenase (ubiquinone) Fe-S protein 2	Ndufs2	Chung	NM_153064	non	NDUFS2 = 1q23	no

transducin-like enhancer of split 3, homolog of Drosophila E(spl)	Tle3	Chung	NM_009389	non	TLE3 = 15q22	no
tachykinin receptor 3	Tacr3	Chung	NM_021382	4q24		Near PARK 4
serine protease inhibitor, Kunitz type 2	Spint2	Chung	NM_011464	19q13.2		no
colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)	Csf2rb2	Chung	NM_007781	non	CSF2RB2 = 22q13.1	no
reticulocalbin 1	Rcn1	Chung	NM_009037	11p13		no
procollagen, type XI, alpha 1	Col11a1	Chung		non	COL11A1 = 1p21	no
FXYD domain-containing ion transport regulator 6	Fxyd6	Chung	NM_022004	11q23.3		no
calbindin 2	Calb2	Chung	NM_007586	16q22.3		no
MARCKS-like 1	Marcks1	Chung	NM_010807	1p35.1		Inside PARK 6
cholecystokinin	Cck	Chung	NM_031161	3p22-p21.3		no
mesoderm development candidate 2	Mesdc2	Chung	NM_023403	non	MESDC2 = 15q13	no
RIKEN cDNA 9130213B05 gene	9130213B05Rik	Chung	NM_145562	non		
RIKEN cDNA 9130005N14 gene	9130005N14Rik	Chung		non		
huntingtin-associated protein 1	Hap1	Chung	NM_010404	17q21.2		no
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	Smarca1	Chung	NM_053123	non	SMARCA1 = Xq25	Inside PARK 12
dedicator of cytokinesis 6	Dock6	Chung		19p13.2		no
CaM kinase-like vesicle-associated	Camkv	Chung	NM_145621	3p21.31		no
isocitrate dehydrogenase 1 (NADP+), soluble	Idh1	Chung	NM_010497	2q33.3		Near PARK 11
nucleobindin 2	Nucb2	Chung	NM_016773	11p15.1-p14		no
ribosomal protein L36a	Rpl36a	Chung	NM_019865	non	RPL36A = Xq22.1	Inside PARK 12
lipoprotein lipase	Lpl	Chung	NM_008509	non	LPL = 8p22	no
adenylate cyclase activating polypeptide 1	Adcyap1	Chung	NM_009625	18p11.32		no
G substrate	Gsbs	Chung	NM_011153	non	C7orf16 = 7p15	no
translocase of inner mitochondrial membrane 10 homolog (yeast)	Timm10	Chung	NM_013899	11q12.1		no
ADP-ribosylation factor-like 5A	Arl5a	Chung		2q23.3		no
asparagine-linked glycosylation 2 homolog	Alg2	Chung	NM_019998	9q22.33		no
calcium channel, voltage-dependent, beta 2 subunit	Cacnb2	Chung	NM_023116	10p12.33		no
carbonic anhydrase 4	Car4	Chung	NM_007607	non	CA4 = 17q23	no
dickkopf homolog 3	Dkk3	Chung	NM_015814	11p15.3		no
dystrophin myotonic-containig WD repeat motif	Dmwd	Chung	NM_010058	19q13.32		no
Eph receptor A6	Epha6	Chung	NM_007938	3q11.2		no
expressed in non-metastatic cells 1, protein	Nme1	Chung	NM_008704	17q21.33		no
glutamate receptor, ionotropic, kainate 3	Grik3	Chung	XM_131647	1p34.3		Near both PARK 10 & PARK6
heme oxygenase (decycling) 2	Hmox2	Chung	NM_010443	non	HMOX2 = 16p13.3	no
histone 3, H2a	Hist3h2a	Chung	NM_178218	1q42.13		no
HIV-1 tat interactive protein 2, homolog (human)	Htatip2	Chung	NM_016865	non	HTATIP2 = 11p15.1	no
mannoside acetylglucosaminyltransferase 5, isoenzyme B	Mgat5b	Chung	NM_172948	non	MGAT5B = 17q25.2	no
RIKEN cDNA 9630033F20 gene DNA	9630033F20Rik	Chung		non		

9630033F20 gene					
tetraspanin 6	Tspan6	Chung		Xq22.1	inside PARK 12