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Minireview

Contribution of genomics and proteomics in understanding the role of modifying factors in Parkinson's disease

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Parkinson's disease (PD) is a complex neurological disorder, characterized by selective degeneration of *nigrostriatal dopaminergic* neurons. It is a multi-factorial disease, contributed by a combination of age, genetic and environmental factors. Etiology of sporadic PD and mechanism underlying selective loss of *dopaminergic* neurons has not yet been clearly understood. Recent developments in genomics and proteomics have revolutionized the research on PD at genetic level. Differential gene expression patterns (DNA biochip technology), age-dependent complex genetic patterns (SNP genotyping), and protein expression profiles (proteomics) of PD patients have started providing the specific and rigorous molecular explanation and role of modifying factors in PD. Genomics and proteomics are further expected to help in developing biomarkers for diagnosis of early onset PD and also to develop valuable and potential therapeutic strategies for its treatment. In this review, we have discussed the progress made by genomics and proteomics, in understanding the role of modifying factors in PD.

Keywords: Parkinson's disease, genomics, microarray, single nucleotide polymorphism (SNP), proteomics

Introduction

Parkinson's disease (PD), a devastating movement disorder associated with aging, is characterized by an abnormal and progressive degeneration of *dopaminergic* neurons (dopamine-producing cells) in *substantia nigra pars compacta* (SNPC) region of mid brain[1,2]. Idiopathic degeneration of *dopaminergic* neurons results in greatly decreased level of dopamine (DA) in the *striatum*. PD is called idiopathic/sporadic, since definite causes of disease are not known. Dopamine, a neurotransmitter, stimulates motor neurons and controls muscular movement and when its production is depleted, motor system nerves are unable to control movement and coordination. The major symptoms of PD are tremor or trembling in several parts of body such as hands, arms, legs, jaw, and face, stiffness or rigidity in the limbs and trunk, bradykinesia or slowness of emotional and voluntary movements and postural abnormality or unstable and impaired balance and coordination. As the disease progresses and severity increases, patient may also show several other symptoms, such as difficulty in walking, talking and performing simple or important tasks, drooling of saliva, chewing and swallowing, festination, depression, emotional changes, memory loss or slow thinking, speech changes, urinary problems, constipation and skin problems[1-8].

PD is the second most common progressive neurological disorder with a prevalence of 0.1 to 0.2 percent[3], however, after the age of 50, incidence rises up to 1-2 per cent. Neurodegeneration often starts

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Abbreviations: AD, Alzheimer disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; CYP, cytochrome P-450; DA, dopamine; DARPP, dopamine and cAMP-regulated phosphoprotein; EGCG, (-)-epigallocatechin-3-gallate, ESTs, expressed sequence tags; GABA, γ aminobutyric acid; GST, glutathione-s-transferase; HFE, haemochromatosis; MAO, monoamine oxidase; MB, maneb; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; NQO-1, NAD(P)H: ubiquinone oxido-reductase-1; NM, neuro-melanin; 6-OHDA, 6-hydroxydopamine; 8-OHDG, 8-hydroxydeoxy guanosine; PCR, polymerase chain reaction; PD, Parkinson's disease; PMNs, polymorphonuclear leukocytes; PON, paraoxanase; PQ, paraquat; R-APO, R-apomorphine, RNS, reactive nitrogen species; ROS, reactive oxygen species; SN, *substantia nigra*; SNPC, *substantia nigra pars compacta*; UPR, unfolded protein response

after the fifth decade of life and progresses over 5-10 years, before reaching the fully symptomatic state. It is more common among men than women[4], though reports are also available mentioning the lack of gender preference[5] and appears to be more widespread in northern countries. Although age is considered as a major contributory factor of PD, many other modifying factors also contribute significantly in onset and progression of disease. Despite extensive research on brain of PD patients or on experimental animal models, etiology of sporadic PD and mechanism underlying selective neuronal loss have not yet been fully understood.

PD is a multi-factorial disease and contributed by a combination of age, genetic and environmental factors[6-8]. Several modifying factors, in combination are involved in the manifestation of PD. These include Caucasian ancestry[9], environmental toxins, herbicide/pesticide exposure[10-12], rural residence, metal exposure[13-23], etc., in addition to higher intake of dietary fats, genetic predisposition, free radicals, accelerated aging, male gender[4], conservative pre-Parkinson's personality and family history. After discovery of involvement of both chemical toxins and genetic mutations[24-27] in PD, efforts are being made to unravel the process that leads to the death of brain cells and ultimately the rigidity, tremors and other symptoms of PD.

In the present review, contribution of genomics, proteomics and recently developed tools of molecular biology, for better understanding of these modifying factors in onset and progression of PD has been described.

Environmental factors

The major modifying factors of PD remain a mystery, however, late onset and slow-progressing nature of PD has prompted the consideration of environmental exposure to agro-chemicals, including pesticides as a risk factor[28]. Early exposure to well water drinking and head trauma may also trigger and expedite the appearance of Parkinsonian features[29]. Etiological study in twins[30] suggested involvement of environmental factors in typical, non-familial PD, beginning after age of 50 years[31]. Epidemiological risk factor analyses of typical PD cases identified contribution of several neurotoxicants, such as N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat (PQ), dieldrin, manganese (Mn) and salsolinol[31].

Environmental toxins lead to the impairment in mitochondrial function in the nerve cells. They affect structure of several genes and increase susceptibility of disease[24-27]. Excessive degeneration of *dopaminergic* system might be the outcome of extended insults by environmental or endogenous neurotoxins in individuals, genetically susceptible to PD. Toxic effects, resulting from excess oxygen damage, combined with exposure to pesticides such as rotenone could act synergistically to cause PD[32]. Some toxicants/drugs analyzed by genomics and proteomics and the relevant genes/proteins/pathways affected by them are given in Table 1.

Heavy metals

Heavy metals help in catalyzing free radical reactions that destroy DA-producing cells and, therefore, implicated as causative agent in onset and progression of PD[13-23]. Individuals exposed to high environmental levels of Mn, such as miners, welders and those living near ferro-alloy processing plants display a syndrome known as manganism, best characterized by debilitating symptoms, resembling PD[33]. Manganese decreases monoamine oxidase (MAO) activity and inhibits the respiratory chain that accumulates in mitochondria and inhibits efflux of

Table 1—Some toxicants/drugs analyzed by genomics and proteomics and the relevant genes/proteins/pathways affected by these toxicants/drugs[13-23,28,33,34,37-39,58,60,61,63,75,91,95,97]

Toxicants and drugs	Relevant genes/proteins/pathway
Heavy metals	Detoxifying enzymes and some antioxidants, such as catalase, superoxide dismutase, glutathione peroxidase and GST
6-Hydroxydopamine (6-OHDA)	Alterations in the expression of genes implicated in oxidative stress, inflammatory processes, signal transduction, glutamate toxicity and apoptosis
Maneb (MB) and paraquat (PQ)	Genes involved in oxidative stress such as catalase, superoxide dismutase, glutathione peroxidase, GST and phase-I and -II detoxification genes
N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)	Genes involved in detoxification pathway, cytoskeletal stability and maintenance, synaptic integrity, cell cycle, antioxidant enzymes, transcription factors, neuronal receptors, growth factors and apoptosis

calcium[34]. Iron is implicated in neuronal damage associated with PD, due to DA and motor disturbances[35,36]. Population exposed to aluminium contamination in the drinking water also has a high risk of developing PD[37-39].

Pesticides

Epidemiological studies showed exposure to agrochemicals as potential environmental risk factor for PD[38,40,41] in older persons that could not be explained by genetic heritability[40]. Variation in PD mortality by geographical regions was also found consistent with an environmental exposure etiology[42-44]. A consistent pattern of high PD morbidity was found among occupational groups employed in agriculture and horticulture in Denmark[45]. Although proportional correlation between the risk of PD onset and pesticide exposure time was reported, no significant dose-response relationship was established[46].

PD is also associated with a systemic defect in mitochondrial complex I activity[47]. Animal models indicated that exposure to inhibitors of mitochondrial complex I, including pesticides, is sufficient to reproduce features of PD. Complex I defects result in oxidative stress that increases susceptibility of neurons to excitotoxic death[32]. The chronic and systemic inhibition of mitochondrial complex I enzyme (nicotinamide adenine dinucleotide ubiquinone reductase) by rotenone, a lipophilic pesticide causes a highly selective *nigrostriatal* dopaminergic degeneration, associated behaviorally with hypokinesia and rigidity[48]. Nigral neurons in rotenone-treated rats accumulate fibrillar cytoplasmic inclusions that contain ubiquitin and α-synuclein[48]. *Striatal* neurons containing dopamine and cAMP-regulated phosphoprotein-32 (DARPP-32) and neurons of the globus pallidus and subthalamic nucleus, however, remain intact with normal morphology[49].

The herbicide N, N'-dimethyl-4, 4'-bipyridylium (paraquat), an environmental/agro-chemical, following inadvertent exposure acts as a modifying factor in onset of PD, due to its structural similarity to MPP^+ (1-methyl-4-phenylpyridinium ion)[50]. Paraquat (PQ) crosses the blood-brain barrier, where higher levels are evident at 24 h after its administration[51]. Direct injection of PQ into brain alters DA level, personal behavior and induces neuronal loss[52-55], however, systemic administration generally does not result neurotoxicity in rodents[52,56,57].

Exposure to pesticides, such as PQ or maneb (MB) during critical periods of development could permanently damage the *nigrostriatal dopaminergic* neurons that enhance its vulnerability to subsequent exposure to neurotoxicants[58-61]. Degenerating cell bodies were observed only in SNPC and *glial* response was found in the ventral mesencephalon, but not in frontal cortex and cerebellum in mice. A significant depletion in *striatal* DA level (rather enhanced DA synthesis) and an increased tyrosine hydroxylase (TH) activity, following PQ administration was reported. The number of GABAergic cells in the *SN pars reticulata* and *hippocampus*, however, remain unchanged. The discrepancy between neurodegenerative and neurochemical effects represent an important feature of PQ model and is probably a reflection of compensatory mechanisms by which neurons that survive damage are capable of restoring neurotransmitter tissue levels[60].

Dithiocarbamate fungicides also possess potent *dopaminergic* activity and diethyldithiocarbamates, a class of dithiocarbamates augmented neurotoxicity of MPTP[61,62]. Dithiocarbamates are reported to alter the kinetics of different endogenous and exogenous compounds and enhance their neurotoxicity[63]. Neurological impairments, resembling PD were also reported in individuals exposed to MB[64,65]. Acute intraperitoneal administration of manganese ethylene-bisdithiocarbamate (maneb) exacerbated attenuation in locomotor activity and augmentation in MPTP-induced catalepsy in mice. Maneb itself inhibits locomotor activity and augments aloperidol-induced catatonia[66,67]. Residual levels of an organochlorine pesticide dieldrin were found in brain of one third of PD patients, as compared with controls[68,69].

Oxidative stress

The increase in occurrence of age-specific prevalence of PD, due to apoptosis and oxidative stress was found consistently with age[70,71]. Involvement of multiple genes and proteins in PD was reported by using neuroprotective and pro-apoptotic drugs[72]. The vulnerability of *dopaminergic* neurons in *substantia nigra* (SN) of PD patients was correlated with the presence of neuromelanin (NM) that acts as an endogenous iron-binding molecule in *dopaminergic* neurons of the SN in human brain[73]. Interaction between NM and iron leads to an increase in indices of oxidative stress in PD[74]. Significant inverse correlation was found between amount of superoxide radicals and specific activities of mito-

chondrial enzymes in PD, and mitochondrial function was significantly affected in both males and females[34].

Genome and proteome-based studies also revealed that oxidative stress played a role in onset and progression of sporadic or chemically-induced PD. Alterations in expression of genes involved in oxidative stress, inflammatory processes, signal transduction and glutamate toxicity was reported in PD[75]. These pro-toxic genes appeared to be compensated by the elevated expression in trophic factors and antioxidant defenses, which were also activated by short exposure to MPTP[75]. The time-course changes in pro-toxic gene expressions indicated importance of early gene cascades occurring prior to late *nigro-striatal dopaminergic* neuronal cell death[75].

Involvement of oxidative stress, inflammatory processes in neurodegeneration and brain gene alterations in MPTP mice model of PD using atlas mouse cDNA expression array is well established[76]. Attenuation in expression of many genes by an iron chelator-radical scavenger drug R-apomorphine (R-APO) supported its neuroprotective role and provided a novel molecular probe in unraveling molecular mechanism(s) of oxidative stress-induced pathogenesis of PD[76]. Further functional characterization of affected genes, including expressed sequence tags (ESTs) would help in predicting complete molecular pathology, and to develop biomarkers for monitoring degenerating dopaminergic neurons in PD[77].

Early-life occurrence of inflammation in the brain as a consequence of either injury or exposure to infectious agents plays a crucial role in pathogenesis of PD[78]. Augmentation in lipid peroxidation and DNA oxidation by-product 8-hydroxydeoxy guanosine (8-OHDG) in SN of PD patients has been found, as compared with age-matched controls[79-82]. Increased level of endogenous 6-hydroxydopamine (6-OHDA) in PD patients indicated involvement of early response genes in oxidative stress-mediated *dopaminergic* cell death. It also revealed involvement of similar mechanisms in the development of PD[83].

Evidence for contribution of oxidative stress also included increased level of iron and decreased levels of ferritin in the brain, especially in the SN and loss of anti-oxidative machinary in PD patients. Increased iron levels were implicated in induction of cytotoxicity through excessive accumulation of hydroxyl radical in PD[84]. A significant increase in basal nitrite content in polymorphonuclear leukocytes (PMNs) of PD patients exhibited an increase in neuronal nitric oxide synthase (NOS) activity in PD[85]. Nitration of tyrosine and increased level of nitrated proteins in brain and cerebrospinal fluid (CSF) of Alzheimer disease (AD) patients demonstrated the potential involvement of reactive nitrogen species (RNS) in neurodegenerative disorders[86].

Proteomics based approaches such as two-dimensional PAGE and mass spectrometry, coupled with immuno-chemical detection techniques are routinely used for the determination of specific targets of protein oxidation in AD brains[86]. Similar approaches may also be developed for PD. Oxidants remarkably induce sequential molecular events such as augmentation in ROS level, activation of JNK MAP kinases, PITSLRE kinase, p110 etc., by both caspase-1 and 3-like activities and apoptotic cell death. Pharmacological intervention using combination of antioxidant trolox and a pan-caspase inhibitor Boc-(Asp)-fmk (BAF) exerted significant neuroprotection against ROS-induced *dopaminergic* cell death[31]. The high throughput cDNA microarray screening was used for the identification of down-stream genes that could be used as biomarkers to monitor ongoing changes in *dopaminergic* neurons under neurotoxic insult[31].

A dose-dependent protective effect of coffee, tea and drinking in PD was reported in an ethnic Chinese population, highlighting involvement of antioxidants in neuroprotection[87]. DA, R-APO, polyphenol (-)-epigallocatechin-3-gallate (EGCG) and melatonin act as neuroprotective and free radical scavengers in PD patients[88]. A concentration and time-dependent correlation between R-APO, DA, EGCG and melatonin in modulation of cell survival/cell death-related gene pathways was also confirmed by quantitative real-time PCR and protein profiles. Unlike the effect of low concentrations of antioxidants (1-10 μ*M*), where an anti-apoptotic response was manifested, a pro-apoptotic pattern of gene expression, such as increase in caspases, fas and gadd 45 was observed at toxic concentrations (50-500 μ*M*) of antioxidants[88].

Genetic factors

In a small number of PD cases, a strong inheritance pattern demonstrated the role of genetic predisposition in PD. Gradual progression of genetically pre-disposed PD was dependent on a trigger such as trauma, other illness or exposure to environmental toxins[24-27]. Several genes and gene loci linked with

familial forms of PD have been identified[24,25]. Familial aggregation and sibling risk studies supported a genetic component for late onset of idiopathic PD[24,25]. Linkage screening provided strong evidence for involvement of multiple genetic susceptibility loci in PD[26]. G88C and G209A mutations in synuclein gene play an important role in the onset of PD, however, unlike the Western population, such mutations were not predominant genetic determinant of PD in the Indians[89].

Genomics, proteomics and PD

Genomics (differential gene expression and complex single nucleotide patterns), along with proteomics are being used for large-scale analysis and characterization of genes and proteins involved in PD[90,91]. Role of detoxification and many other genes and proteins have been elucidated in PD using these sophisticated tools. Single nucleotide polymorphism (SNP) in antioxidant and detoxification genes determines the susceptibility of an individual towards onset of PD. The advent of novel tools of genomics and proteomics during the past decade has resulted in differential expression profiles of thousands of genes and proteins, involved in the degeneration of DA-containing cells in PD and has allowed more focused treatment of PD according to individual genotypes[90,91]. Development of high throughput technologies, such as microarrays for gene expression has led to innovative approaches in elucidating onset of PD, reflecting the entire spectrum of molecular mechanism of sporadic PD. Identification of SNP in the most relevant genes involved in onset and progression of PD and establishing its association with population of varying ethnic groups is expected to help in the development of individual specific medicines for treatment of PD. The combined approaches of mRNA expression profiling (transcriptomics), SNPs and protein expression patterns (proteomics) in the relevant genes have been used to understand the role of gene-environment interactions in PD[90,91].

Transcriptomics

Transcriptomics is the study of a transcriptome, which is defined as a complete set of transcripts expressed by the entire genome at a given time. Microarray is used extensively to study transcriptome to analyze the differential gene expression between the cells, tissues, organs or populations of varying biological status or exposure conditions. Recent developments in molecular technologies and bioinformatics permit the rapid assay and interpretation of several thousand gene transcripts, involved in PD on small glass or siliconated slides or chips.

DNA microarrays (cDNA and oligonucleotides based) can be used to study and identify the mechanisms of neurodegeneration and neuroprotection in which global expression of thousands of genes can be assessed simultaneously. cDNA microarrays-based differential gene expression in PD has been proved as a powerful tool for mechanistic assessment of toxic response. Thus, differential gene expression profiles identify samples exposed to toxicants, to predict toxicity of unknown compounds in brain tissues and also to study their cellular mechanism. This technology may also be used to develop effective neuroprotective drugs[28,76,77,88,90,92] and could be conveniently applied to solid tissue samples, such as liver, skin and renal biopsies. The major advantage of this approach is sensitivity, as very low levels of transcripts can be measured. Due to limitations of biochemical methods in the global analysis of neuronal death, a full picture of events has not yet been established[93]. However, cDNA microarray or microchip is changing the prospects for understanding the disease process, its progression and response to drugs, etc.[93]

cDNA microarray has been extensively used in drug discovery and design, for identifying the 'fingerprints' as potential targets for drug intervention[90]. In neurodegenerative disorders, glutamate receptor 2 gene was up-regulated and transcription-associated genes got down-regulated in non-human primate (NHP) models, compared with humans. This has provided transcript profiling of NHPs for comparative genomic data to validate and focus experimental animal models of human neurological disorders[94].

Early gene expression profile in the SN and *striatum* of acute MPTP-induced PD in mice revealed alteration in a restricted number of genes affected by the long-term MPTP treatment. Differential gene expression profiles examined MPTP-induced *nigrostriatal dopaminergic* neuronal degeneration and its protection with R-APO using cDNA microarray (comprising 1,200 different gene fragments)[28]. Around 50 genes were found altered in MPTP-induced PD. cDNA microarray technique was also used to study gene expression to determine the mechanism of action of MPTP in mouse SN. In

addition, it was used to study the neuroprotection induced by several compounds, including R-APO and EGCG[93]. Acute exposure of MPTP induced an early response gene expression, whereas chronic exposure directly induced the genes, usually expressed at the time of death. Early gene changes are crucial for setting into action genes that eventually cause *dopaminergic* neuronal death[95]. The neuroprotective drugs reversed some of expressed genes, suggesting involvement of early response genes in the neurodegenerative process[93].

The array data of 6-OHDA-mediated PD suggested that DA denervation of the *striatum* resulted in impairment in DA-protein kinase A-cyclin dependent kinase 5-protein phosphatases cascade which regulated the state of phosphorylation and activity of DARPP-32[90,91]. PKA and CD5 genes regulate DARPP-32, a mediator of DA signaling by phosphorylating specific sites at thr-34 and thr-75. Protein phosphatases PP1 and PP2A play a pivotal role in dephosphorylation of phospho-DARPP-32 (target of PKA) at thr-75 site. 6-OHDA-induced denervation resulted in modulation of expression of genes encoding for components of DA signaling network. Denervation modulated expression of genes that target DARPP-32, as an integration crossroad for intra-cellular DA signaling[96].

cDNA microarray gene expression profiling indicated that mechanism of neurodegeneration by MPTP and 6-OHDA is a complex cascade of several biochemical and molecular events[91,95]. Alteration of genes associated with iron metabolism, supported oxidative stress-induced neurodegeneration, involving iron deposition in SNPC[95]. MPTP and R-APO-induced alterations in 49 different genes, involved in oxidative stress, inflammation and transferrin receptor genes such as oxidative stress-induced protein A 170, cytochrome P4501A1, Osp94, cytotoxic cytokines, IL-1, IL-6, TNF-α, IL-10, glutamate receptors, N-methyl-D-asparate (NMDA), glial cell derived neurotrophic factor (GDNF), epidermal growth factor (EGF), and NOS, but not α amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor genes[28]. An additional cascade might act in parallel to oxidative stress and inflammation to converge into a common pathway, leading to neurodegeneration. R-APO prevented overexpression of several genes that participate in cell death[28].

As series of events occur in neurodegeneration, a single drug may not be adequate to induce neuroprotection and thus use of cocktail of drugs may be more appropriate[90]. 6-OHDA-induced DA denervation in the nucleus of *striatum*[97] caused the modulation of 50 different genes involved in several cellular functions. Experimental genetic manipulation in the genes involved in intra-cellular transduction of DA signal and in regulation of glutamate transmission in *striatal* neurons provides the information on possible neuronal events which lead to reorganization of glutamate transmission in *striatum* of Parkinsonian rats[97]. Subtractive cDNA libraries and microarray analysis have been used to identify the gene expression profile that regulated tolerance to hypoxia in surrounding tissues during transplantation of DA-secreting cells for treatment of PD[92]. An improved understanding of molecular basis of hypoxia tolerance may lead investigators to engineer cells that could survive in hypoxic environment of brain parenchyma, following transplantation[92].

Microarray technology could not be used satisfactorily, for biological fluids (blood and body fluids), since it is very difficult to isolate good quality mRNA from these samples. The measurement of transcript levels could not provide complete information about functionally important proteins and their role in onset of PD. Therefore, further information could be gained from protein profiling.

Genetic mutations and SNP

Genetic mapping approach in rare familial cases, with autosomal recessive and dominant inheritance of PD suggested wide genetic heterogeneity of disease. α-Synuclein (park 1), parkin (park 2), N-acetyl transferase 2, tau, ubiquitin C-terminal hydrolase-L1 (UCH L-1, park 3), DJ-1 (park 4) and NAD(P)H: ubiquinone oxido-reductase-1 (NQO-1) genes are involved in onset of familial PD. Mutations in genes altered expression/activity of proteins, leading to loss of their normal function. Gene loci allowed a specific clinical investigation of affected families to study clinical heterogeneity of PD. Identification of mutations in α-synuclein, parkin and ubiquitin C-terminal hydrolase-L1 involved in protein degradation and aggregation in familial PD initiated search for other genes, involved in pathogenesis of PD[97,98].

Synuclein gene contributes to both common late onset and rare early onset forms of disease while parkin gene is responsible for autosomal recessive juvenile Parkinsonism, a disorder similar to, but considered distinct from PD[89,97,98]. Parkin gene

mutations were also found in families with both late- and early onset cases of PD, with autosomal, recessive juvenile Parkinsonism and intron 7 having the strongest association with early onset of PD[27,99,100]. Parkin gene appears to work in concert with ubiquitin, in ridding the cells of metabolic wastes, suggesting that it might eventually be a useful diagnostic tool for the disease.

Several mutations were found in α-synuclein[31] (4q21.3-23), parkin (6q25.2-27) and ubiquitin carboxy terminal hydrolase-L1 (4p16.3) genes in families with PD[27,99,100]. In families with late onset PD, strongest linkage was found on chromosome 17 near tau gene. Evidence for genetic linkage of PD to five distinct regions on chromosomes 5, 6, 8, 9 and 17 is well documented[27,99,100]. PD showed defects in mitochondrial complex I, encoded by the genetic material of mitochondria[47,70]. Four additional chromosomal locations — 2p13, 4p14-15, 1p35-36, and 12p11.2-q13.1 have been linked with the families suffering from PD.

Involvement of α-synuclein, parkin and ubiquitin C-terminal hydrolase-L1 genes in PD suggested its association with ubiquitin-proteasome dysfunction and aberrant protein degradation. Common DNA-polymorphism including promoter polymorphism or genetic variation in the parkin gene did not contribute to the risk of developing PD[101]. Substitution of valine (V) to glutamate (E) at 56 (V56E) and cysteine (C) to tyrosine (Y) at 212 (C212Y) position, deletion of exons 3, 5 and base A at 225 codon in park 2 gene in the families with recessive inheritance is also reported in pathogenesis of PD[102]. Study indicated variability in clinical phenotype and molecular defects in hereditary PD, due to heterozygous mutations in the genes involved in Parkinsonism[102]. But, no significant association ($P>0.05$) was reported between PD and parkin SNP alleles or genotypes by haplotype analysis and stratification by age at onset or by family history, although parkin mutations were involved in early and late onset of PD[103].

Two single-nucleotide polymorphism within the parkin core promoter were identified[104]. One of the variants 258 T/G located in a region of DNA binds to nuclear protein from human SN and functionally affects gene transcription and is genetically associated with idiopathic PD[104]. Recessive loss of parkin gene is a risk factor for juvenile and early onset of Parkinsonism; however, in some cases, its haplo-insufficiency may be sufficient for disease[105]. The human tau gene, which promotes assembly of neuronal microtubules, was associated with Parkinsonian features and several other rare neurological diseases. It is a susceptibility factor for idiopathic PD[106]. A list of genes significantly associated with onset and progression of various forms of PD are summarized in Table 2. Toxicant responsive genes in which occurrence of SNP is reported to determine susceptibility and severity of PD are listed in Table 3.

T131C substitution at codon 44 in exon 3 of synphilin-1 gene, studied in a Japanese population revealed lack of association with genetic susceptibility to sporadic PD[107]. Variation in haemochromatosis (HFE) gene, an important regulator of cellular iron homeostasis results in iron overload, and was responsible for hereditary HFE[106]. Iron homeostasis was found altered, following cysteine 282 tyrosine (C282Y) conversion due to SNP, in two separate cohorts of Australian population[108]. The role of mutations in HFE gene in PD was proposed, based on C282Y gene mutation; however, rarity of this genotype requires a large series of patients to prove the assumption[109]. This study showed the role of C282Y SNP in protection against the development of PD[108]. Polymorphism in brain-derived neurotrophic factor (BDNF) gene along with the S18Y polymorphism in UCH-L1 gene occurred more frequently in PD patients[110]. This study provided genetic evidence, supporting a role for BDNF in pathogenesis of PD.

Microarray analysis of RNA, isolated from neurotoxins 6-OHDA and MPP^+-treated samples revealed dramatic up-regulation of stress-induced transcription factor CHOP/Gadd153 by both 6-OHDA and MPP^+. MPP^+ increased the phosphorylation of unfolded protein response (UPR) such as PERK and eIF2-α, however, 6-OHDA increased phosphorylation of c-Jun (a protein product of c-Jun gene, involved in apoptosis and neurodegeneration). Involvement of UPR in these widely used toxin models also supported the role of ubiquitin-proteasome pathway dysfunction in PD. 6-OHDA triggered multiple pathways associated with UPR, whereas MPP^+ exhibited a more restricted response[111].

Many enzymes such as CYP system, flavin monooxygenase system, esterases, paraoxonases (PONs) and glutathione-s-transferases (GSTs) metabolize pesticides and are usually involved in initial metabolism of pesticides, leading to either their activation or inactivation[112,113]. As pesticides are a contributory factor in onset of PD, therefore, genetic

Table 2—A list of some genes involved in early or late onset of dominant and or recessive form of PD[27,89,99,100,103,104,106,110,132]

Gene	Chromosomal localization	General features
α Synuclein (Park 1)	Four	Associated with rare cases of early onset PD, discovered in a very few families, follow an autosomal dominant pattern of inheritance
Parkin (Park 2)	Six	Associated with early onset of disease, frequently found in families with PD, follow autosomal recessive pattern of inheritance
Ubiquitin C- terminal hydrolase-L1 (UCH L-1, Park 3)	Four	In families where more than one person had PD, a single brother-sister pair is known to have variants of this gene, associated with early onset of disease, follow autosomal dominant pattern of inheritance
DJ-1 (Park4)	One	Mutations in DJ-1 gene described in autosomal recessive PD patients (ARPD) of European ancestry and young onset (YOPD) of Ashkenazi Jewish and Afro-Caribbean patients. Pathogenic DJ-1 mutations restricted to certain populations associated with early onset of the disease, very unlikely to be of clinical importance in Asian population
Tau	Three	Associated with an increased risk of PD, if expressed in homozygous condition (tau H1). H1 haplotype more efficient than H2 haplotype, follow autosomal dominant pattern of inheritance
N-Acetyl transferase 2	Eight	Slow processing variants found twice as often in persons with familial PD, compared with fast processing variants and known as late onset-related gene
NAD(P)H: ubiquinone oxido-reductase gene 1 (NQO1)1	Mitocondrial DNA	Suspected to be associated with late onset of disease. Mitochondria of brain of PD patients, activity of "complex I" diminishes, however, it is not clear whether it is a cause or a result of the disease
Unknown gene	Two	Found on the long arm of chromosome 2 in some families suffering from PD and is involved in late onset of PD

Table 3—A list of toxicant responsive genes in which occurrence of SNP is reported to determine susceptibility and severity of PD[11,113,114,119,121,122,123,129,130]

Gene	General features
CYP2D6	Involved in detoxification of environmental chemicals/toxicants and significantly associated with onset of PD in Australian population
CYP2E1	CYP2E1 mutations have been hypothesized, however, significant association of polymorphism in this gene with onset of PD in both early and late onset in the Chinese and Taiwanese population is not found
GST	Association between polymorphism in the pi class GST and PD in the subjects reported with pesticide exposure is well-known
NOS	Association between polymorphism in NOS gene and PD in community-based case control studies are known
PON1	Association between population-specific allele frequencies in PON1 gene with PD is known in Asians; however, no significant association is found in Caucasians

variability in these enzymes would be expected to alter the risk of PD by influencing deposition of pesticides. CYP2D6, a toxin metabolizing regulatory gene is involved in the detoxification of environmental chemicals and toxicants[113,114]. A significant association between the polymorphism of this gene with onset of PD, was reported in Australian population[11].

Genetic susceptibility to neurotoxins is one of the major causes of PD and polymorphism in various forms of CYP genes such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 was extensively studied. A significant association of PD with CYP2D6 gene was found in several studies, though inconsistent[115]. Significant genetic polymorphism in CYP2D6 genes in Indian population was reported[116,117], however, evidence is needed to support their role in the onset and progression of PD. The metabolism of deprenyl by deprenyl-metabolizing CYP system in monkey liver microsomes indicated the relevance of CYP-dependent metabolism and its contribution in neuroprotection also in primate models of PD[118]. Genetic polymorphism of CYP2E1 gene and susceptibility to PD was not found significantly related in Taiwanese population[119].

Investigators have also supported the hypothesis that CYP2D6 gene is not a major gene responsible for PD[120]. Role of gender, age at onset and environmental risk in the frequency of CYP2D6-deficient alleles in the patients with PD was also suggested in European population[5]. A CYP2D6 type 4 allele was not associated with earlier PD onset, although its association with survival was reported[115]. In the Chinese popula-

tion, CYP1A1 gene polymorphism was found to be a genetic susceptible factor for early onset of PD, but not CYP2E1 RsaI and PstI polymorphism for both early and late-onset of PD[121]. Genotyping of CYP genes such as CYP2D6, CYP3A4, CYP2C9 and NQO-1 indicated their involvement in chemically-induced PD and helped in understanding the involvement of toxicants in pathogenesis of PD[112].

An association between polymorphism in pi class GST and PD, in the subjects with pesticides exposure[122,123], is of particular interest, because diminished glutathione in the SN is an early finding in PD[124]. GST-O1 gene was also found to be involved in the post-translational modification of inflammatory cytokine interleukin-1β[125,126]. The expression of its isoforms was also reported in blood-brain barrier[122,123]. An association between PON1 gene and PD was found in Asians[127], but no significant association was reported in Caucasians[128].

Population-specific allele frequencies in PON1 gene and its association with onset of PD were also demonstrated[129]. PON1 gene is involved in the metabolism of oxidized lipids and plays a major role in the metabolism and detoxification of insecticides, processed through cytochrome P450/PON1 pathway[129]. An association between polymorphism in NOS gene and PD was also found, thus elucidating the role of genetic polymorphism in PD[130]. Polymorphism in the genes involved in detoxification of drugs/toxins, oxidative stress, neuronal transmission etc. could be responsible for the propensity for PD, progression and efficacy of pharmacotherapy of disease[131]. SNPs in many genes control the onset and progression of PD; a suggested possible mechanism is presented in Fig. 1.

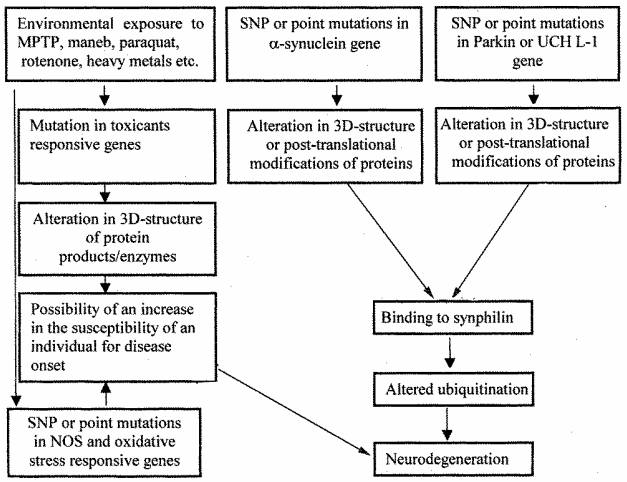

Fig. 1—A SNP in any gene involved in onset of PD results to an altered biosynthesis/pre-mature termination or altered post-translational modification of proteins.Single base mutation in the genes may lead to a change in the sequence of proteins/enzymes and increases/decreases functional response of these proteins for neuronal toxicity. Some neurotoxins may directly act on the α synuclein, toxicant responsive genes, NOS and antioxidant enzymes[11-13, 15-23,27,31,50,52-57,61,62,89,99,100,103,104,113,114,121-123,140,141]

Proteomics

Proteomics is study of gene expression at protein levels and post-translational modification in expressed proteins. Measurement of such changes at protein level is being used for diagnostic purposes. Unlike transcriptomics, in proteomics, all the mechanisms could be identified at cellular level, therefore, study of proteome wide analysis is of major importance. Using two-dimensional (2D) gel and mass spectrometry to measure simultaneously a number of proteins offers the possibility of identifying protein signatures of toxicant exposure.

Proteomics-based approaches have been used to identify biomarkers of toxicant to monitor therapeutic and toxicological responses in PD. The use of proteomics has many important applications for establishing relationship between toxic effects and protein molecular markers. Proteomics is used for identification of toxicological biomarkers, recognition of toxicity patterns and toxicant structure activity (toxicity) relationship. In addition, it offers several potential benefits over the traditional methods. For example, using proteomics, toxic effects could be measured even at lower doses of toxicants that are not possible with conventional methods used in histology and clinical biochemistry.

Proteomics, in combination with genomics would allow researchers to obtain a precise molecular description of a biological sample, to identify differences associated with common PD or PD phenotype or administration of anti-PD drugs. It can also provide powerful strategies for characterization of mitochondrial proteins. Current approaches to mitochondrial proteomics include creation of detailed catalogues of protein components in a single sample or identification of differentially expressed proteins in diseased or physiologically-altered samples.

The opportunities for identification of proteins directly involved in diseases associated with or caused by mitochondrial dysfunction are compelling[70]. Linking genomic array information to actual protein levels in mitochondria may lead to the development of new pharmacological targets of PD[70]. Protein

Table 4—Parameters/biomarkers used for diagnosis or expected to be developed by genomics and proteomics for timely diagnosis of PD[1-8,27,89,97-100,101,103,104,106,115,124,129]

Parameters already used for diagnosis	Biomarkers expected from genomics and proteomics
Microscopic examination for presence of extra-cellular pigmented granules, leading to uptake within macrophages or presence of cytoplasmic inclusion bodies called Lewy-bodies	Peripheral blood protein biomarkers
	Cerebrospinal protein biomarkers
Brain imaging with radioactively-labeled drug, for example fluoro-dopa PET imaging to check impaired fluoro-dopa uptake in the region of the caudate and putamen	Determination of individualistic susceptibility by assessing SNP in parkin, α synucelin, UCH L-1, DJ-1 and other genes involved in PD
Presence of basic symptoms such as bradykinesia, tremor, rigidity, postural abnormality, festination, micrographia etc.	Determination of susceptibility by assessing mutations in phase I and -II detoxification genes
Computerized testing of memory, attention, motor speed, judgment, handwriting and assessment of speech and smell	Signature fingerprints by microarray analysis

biomarkers may be investigated in biological samples obtained using non-invasive methodology and once a biomarker protein or a group of proteins is identified, standard methods, such as immunoassays could be used for screening. Biomarkers expected to be developed by genomics and proteomics for timely diagnosis of PD are given in Table 4.

α-Synuclein, a pre-synaptic protein was found to be the major component of the Lewy-bodies in both inherited and sporadic forms of PD. Its involvement in pathogenesis of nigral degeneration in PD is not completely understood. However, interaction of normal and mutant α-synuclein with the mitochondrial complex IV enzyme cytochrome C oxidase suggested that α-synuclein aggregation (characteristic of mutant) enhanced the mitochondrial dysfunction, which is responsible for onset of PD. Other proteins or genes expressed in brain at time of onset and progression of PD are: Pael-R, α Sp22CDC, rel-1, synphilin-1, UCH-L1 and DJ-1[132].

Transcription of α-synuclein associated genes in age-matched tau transgenic *Drosophila* followed distinct pathways of neurodegeneration[133]. Evidence was also provided for involvement of lipids in PD, by yeast two-hybrids (a proteomics tool used for protein-protein interaction) based studies[134]. Yeast cell manipulation provided an opportunity to dissect molecular pathways, underlying normal and pathogenic (expressed during PD) α-synuclein folding patterns. α-Synuclein expressed in yeast was associated with the plasma membrane in a highly selective manner and inhibited phospholipase D-induced lipid droplet accumulation, thereby affects vesicle trafficking[135]. Besides *nigrostriatal* tissues, CSF is also frequently used as a sample for detecting and monitoring disease biomarkers[136-139]. Since, it is quite difficult to get the neuronal tissues and CSF of human being, therefore, most of the studies are restricted to animal models only.

Toxicogenomics based analysis of PD has provided new insights into the molecular events involved in dose-dependent neuroprotective and toxic activities of neurotoxins[88]. Microarrays and proteomics may provide new insights in understanding the underlying mechanism of PD, which is not feasible with conventional biochemical procedures, as well as new prospects to develop effective therapeutic approaches for the treatment of PD. Even after advent of new powerful tools such as genomics, proteomics, brain imaging, gene replacement therapy and knockout animal models, complete mechanism of PD and its neuroprotection is not yet achieved[90].

Conclusions

PD is a multi-factorial disease and is most likely determined by genetic and environmental factors. Age is a consistent risk factor and an age-dependent cumulative insult could be responsible for selective degeneration of *nigrostriatal* neurons. Although several types of treatments are available to substantially alleviate clinical symptoms of PD, it could not be cured permanently using current therapeutic strategies. Age-dependent complex patterns and genetic expression profiles of degenerating neurons might elucidate the mechanism of onset and progression of PD. Genomics and proteomics tools may lead to the prediction of drug response and might help in the development of potential personalized therapeutic strategies.

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