1. INTRODUCTION

In a monograph entitled “An Essay on the Shaking Palsy” published in 1817, the British Physician James Parkinson presented his observations of six individuals with “paralysis agitans”. Martin Charcot who coined the modern label Parkinson’s disease (PD) later gave us a clear understanding of this syndrome including tremor, slowed movements and gait impairment. A German Neurologist Friedrich Lewy in 1912 described a primary neuropathological lesion later called Lewy Body. During 1950’s a Swedish scientist Advid Carlsson identified the underlying biochemical changes in the brain. In 1967 Cotzi introduced L-dopa, a gold standard drug into clinical practice and the first study about the improvements in patients with PD resulting from treatment with L-dopa was published in 1968.

Parkinson’s disease is a progressive neurodegenerative disorder characterized by development of tremor, bradykinesia, rigidity and postural instability. It is also accompanied by a host of non motor manifestations. PD is predominantly associated with progressive degeneration of dopaminergic neurons in the Substantia Nigra region of the brain (1). The function of Substantia Nigra is to control the movement. It sends both excitatory and inhibitory massages mediated by dopamine to the caudate nucleus and putamen which are responsible for regulatory movements (2). The dopamine deficiency causes dysfunction of basal ganglia by blocking massage transmittance from Substantia Nigra par compacta.

The basal ganglia are located bilaterally in the midbrain and are comprised of a number of subcortical nuclei such as the striatum (caudate and putamen) and the globus pallidus. The basal ganglia are involved in the complex motor movements, associative learning, planning, and emotion which are all mediated by the dopaminergic system. Basal ganglia are also involved in the complex connections with numerous other parts of the brain. Due to this type of mechanism, degeneration of Substantia Niagra pars compacta creates a shortage of dopamine, leading to symptoms like tremor at rest, bodily rigidity, and marked slowness of movement. Lewy bodies are formed due to formation of insoluble protein. This is a pathological hallmark of PD.

Parkinson’s disease is also called as a movement disorder, which is both chronic and progressive in nature. It is characterized by muscle rigidity, tremor, bradykinesia and
akinesia. The primary symptoms are the results of excessive muscle contraction, normally caused by the insufficient production and action of dopamine, which is produced by the dopaminergic neurons of the brain. Secondary type of symptoms include high-level cognitive dysfunction and subtle language problems.

PD occurs due to Parkinsonism, characterized by a group of similar symptoms. It is also called "primary parkinsonism" or "idiopathic PD" ("idiopathic" means of unknown cause). Idiopathic form of Parkinsonism is common. There are some cases where the symptoms result from toxicity, drugs, genetic mutation, head trauma, or other medical disorders.

1.1 Genetics of Parkinson's disease

Etiology of Parkinson's disease is varied in nature. About 10% of the cases are thought to have genetic origin and rest 90% are sporadic in nature. The genetics of Parkinson's disease follows Mendelian type of inheritance. Several genetic mutations have been identified in patients with familial forms of the Parkinson's disease. Mutations in two genes are involved in Parkinson's disease namely, Alpha Synuclein gene and Parkin gene (3). Familial and idiopathic PD are clinically not very clearly distinguishable. But however, the patients with familial disease show some features that are not found in patients with idiopathic Parkinson's, such as, early age of disease onset which ranges between 30-60 years (4). The presence of the classical symptom trait of the disease, such as resting tremor, rigidity, bradykinesia in addition to good response to levodopa therapy are treated as preliminary distinguishable characteristics of Parkinson's disease(5).

The exact pathogenesis and etiology of PD is still unknown. Recent work suggests that some mutations are responsible Parkinson's disease (6). In Parkinson's disease mutations have been identified in genes like SNCA (α-synuclein), PINK1 (PTEN-induced putative kinase 1), Parkin, UCH-L1 (ubiquitin C-terminal hydrolase L1), DJ-1 (PARK7), NR4A2 (nuclear receptor subfamily 4, group A, member 2) and LRRK2 (leucine-rich repeat kinase 2) (7). Recent studies show that there is association of some more gene loci (PARK3, PARK4, PARK6, PARK8, PARK9 and PARK10) which are still to be elucidated functionally (8). Genetic mutations in genes like Parkin, PINK1 and
Molecular Characterization of SNCA Gene (α-Synuclein) in Parkinson's Disease

DJ-1 are autosomal recessive, which cause early onset familial PD (9), while mutations in SNCA and LRRK2 are autosomal dominant.

SNCA (α-synuclein) gene plays a very important role in the pathogenesis of PD. SNCA gene is located on chromosome number 4q21 and has 6 exons. In SNCA gene three types of point-mutations (A30P, E476K and A53T) have been identified, as well as gene duplications and triplications have also been observed (10). Although SNCA mutations are seen in only 1% of PD cases, aggregation of the SNCA protein α-synuclein is identified in all patients suffering from PD. Furthermore, in Lewy bodies α-synuclein protein is present. It is one of the pathological characteristics of PD (11).

Figure 1: Chromosomal location of SNCA gene

In Parkinson disease, mitochondrial dysfunction plays a vital role in neuronal cell loss and the development of PD. There are two genes which are associated with mitochondrial dysfunction in PD: viz PINK1 and Parkin (PARK2) (12). PINK1 protein consists of serine/threonine kinase, which is located in the mitochondrial inner membrane and is important for maintaining mitochondrial homeostasis within dopaminergic neurons. PINK1 is able to directly phosphorylate parkin, an ubiquitin protein ligase that is related to the proteasome. After phosphorylation, parkin regulates mitochondrial function. It is not completely understood as to how PINK1 can phosphorylate parkin, which is located in the cytoplasm. Probably, PINK1 uses a signal transduction pathway to stimulate parkin to ubiquitinate its targets, which in turn affects mitochondrial function (13). Parkin is a largest gene in which over 70 PD associated mutations have been identified. Familial PD associated mutations in parkin disturb its ubiquitin ligase activity.
Parkin gene is associated with PD vary from Parkinsonism to severe dystonia. Parkin and α-synuclein genes are associated; and both are found in LBs (15).

The UCH-L1 protein (ubiquitin carboxyl-terminal esterase L1; PARK5) functions in between the ubiquitin-proteasome system (UPS), the endosomal-lysosomal pathway and LBs which are present in late-onset PD. UCH-L1 is present in the brain and manages a stock of mono-ubiquitin for the UPS (16). Furthermore, UCHL1 gene also stimulates the preservation of free ubiquitin in the endosomal-lysosomal pathway. Hence mutations in the UCH-L1 gene result in impaired functioning of the endosome-lysosome pathway and the UPS system, resulting in LB formation (10). In UCH-L1 gene, I93M mutation is associated with dominantly inherited PD. Besides, UCH-L1 gene I93M-transgenic mice show progressive dopaminergic cell loss in the substantia nigra. This implicates that an I93M mutation in UCH-L1 gene plays important role in development of PD (17).

Although DJ-1 (PARK7) was first identified as an oncogene, it also plays a vital role in the development of PD (18). Furthermore, DJ-1 gene occurs in other types of neuropathological diseases, including stroke (19). Early onset PD usually develops due to DJ-1 gene mutations that is autosomal recessive in nature. Severity and slowness of disease is caused by DJ-1 mutation (20). Till date the exact role and functioning of DJ-1 protein in PD remains unclear, as well as the mechanism of DJ-1 causing dopaminergic cell death (21). However, this gene also exhibits initiators of oxidative stress, among which dopaminergic toxins rotenone (a chemical used as pesticide that interferes with the mitochondrial electron transport) and MPTP (1-methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine) a toxin functions on the substantia nigra and causes neuronal cell death (22). These functions give the indication that DJ-1 mutations play an important role in the development of PD.

NR4A2 (nuclear receptor subfamily 4, group A, member 2) gene encodes a transcription factor regulating, among others tyrosine hydroxylase, which is important for the differentiation and maintenance of dopaminergic neurons. Therefore, a decrease in NR4A2 in the brain increases the vulnerability of dopaminergic neurons to stress and might thus play a role in the pathogenesis of PD (23). The phenotype of NR4A2-linked Parkinsonism causes late-onset PD (24).
LRRK2 mutations occur in 2% to 6.4% of familial PD cases and 0.6% to 3.4% for idiopathic PD cases. LRRK2 gene is located in LBs and potentially regulates both α-synuclein and parkin by functioning as a kinase. The LRRK2 protein is encoded by the LRRK2 gene Therefore, LRRK2 plays a vital role in LB formation and PD pathogenesis (25). The detail mechanism of neuronal death in Parkinson disease is studied by the protein misfolding cyclic amplification (PMCA) technique. PMCA is a fast and reproducible system that can be used as a high throughput screening method for finding new α-synuclein anti-aggregating compounds (26).

1.2 Symptoms of Parkinson’s disease

The symptoms of Parkinson’s disease can be classified as motor symptoms and non-motor symptoms. The motor symptoms include tremor, rigidity, bradykinesia and postural instability, whereas non-motor symptoms include disorders of mood, behavior, thinking and sensation. Individual patient’s symptoms may be quite dissimilar and the progression also distinct based on the individual genetic makeup.

Motor Symptoms: There are two types of motor symptoms in PD
A. The cardinal/primary motor symptoms of PD are
i. Tremor: This is the most common symptom of PD. It is estimated that 30% of patients have little perceptible tremor. In tremor, there are two types, resting tremor which occurs when the patient is sitting or lying still. Resting tremors often affect only the hands or fingers. Another type is action tremors which occur during movement of the affected body part.
ii. Rigidity: Rigidity is also referred to as resistance to movement. It affects most parkinsonian patients, Stiffness; increased muscle tone; in combination with a resting tremor, produces a ratchety, also referred as "cogwheel" rigidity which means the limb is passively moved.
iii. Bradykinesia/akinesia: Bradykinesis, is the slowing down and loss of spontaneous and automatic movement, slowness or absence of movement. Rapid, repetitive movements produce a dysrhythmic and decremental loss of amplitude. Also "dysdiadokinesia", is the loss of ability to perform rapid alternating movements.
iv. Postural instability: It is failure of postural reflexes, which leads to impaired balance and falls.

Above said symptoms like tremor, rigidity, and slowness of movement occur due to depleted dopamine levels in substantia nigra neurons. Where as in normal individuals, a signal is sent down to neurons from an area of the brain called the SN, and is received in the striatum. Messages cross the gap in between the neurons, which act as chemical messenger for dopamine. In people affected with PD the cells in the SN produce much less dopamine, so this pathway is blocked, leading to the appearance of above symptoms.

B. Secondary symptoms
i. Gait and posture disturbances

Shuffling gait: It is characterized by short steps, with feet barely leaving the ground, producing an audible shuffling noise. Small obstacles tend to trip the patient.

Decreased arm swing: It is a form of bradykinesia; turning "en bloc": rather than the usual twisting of the neck and trunk and pivoting on the toes, PD patients keep their neck and trunk rigid, requiring multiple small steps to accomplish a turn. Stooped, forward-Flexed posture: In severe forms, the head and upper shoulders may be bent at a right angle relative to the trunk (camptocormia).

Festination: a combination of stooped posture, imbalance, and short steps. It leads to a gait that gets progressively faster and faster, often ending in a fall.

Gait freezing: "Freezing" is another word for akinesia, the inability to move. Gait freezing is characterized by inability to move the feet, especially in tight, cluttered spaces or when initiating gait.

Dystonia (in about 20% of cases): It is a secondary type of symptom where we can observe abnormal, sustained, painful twisting muscle contractions, usually affecting the foot and ankle, these were characterized by toe flexion and foot inversion, interfering with gait. Whereas, dystonia is quite common, which involves a majority of skeletal muscles; such episodes are acutely painful and completely disabling.

ii. Speech and swallowing disturbances

Hypophonia: Speech quality tends to be soft, hoarse, and monotonous. Some people with Parkinson's disease claim that their tongue is "heavy". Festinating speech:
excessively rapid, soft, poorly intelligible speech. **Drooling**: most likely caused by a weak, infrequent swallowing of saliva and stooped posture.

**Dysphagia**: It is the impaired ability to swallow. It can lead to aspiration, pneumonia, and ultimately death.

**Non-Motor Symptoms**

i. **Mood disturbances**

Depression is one of the most common mood disturbances that occur in patients with PD. The prevalence rates of depression are estimated vary widely according to the population size and methodology used. The occurrence rate of depression is estimated to vary from 20-80% of cases Estimation from community samples will give lower rates than from specialist centers (27).

Most of the studies done so for by using self-report questionnaires such as the Beck Depression Inventory, gives high scores due to physical symptoms. Studies done by using diagnostic interviews by trained psychiatrists report lower rates of depression. 70% of individuals with PD will be diagnosed with preexisting depression going on to develop anxiety. In about 90% of PD patients pre-existing anxiety subsequently develops into depression; apathy or abulia (28).

ii. **Cognitive disturbances**

Cognitive disturbance is present to some degree in most Parkinson's patients. It may lead to Dementia. Initially it will show slowing of thought and later it may lead to difficulties in abstract thinking, memory, and behavioral regulation. Slowed reaction time: both voluntary and involuntary motor responses are significantly slowed executive dysfunction-characterized by difficulties in, differential allocation of attention, impulse control, set shifting, prioritizing, evaluating the salience of ambient data, interpreting social causes, and subjective time awareness (28).

iii. **Sleep disturbances**

In PD more frequently observed sleep disturbances are disturbances in REM sleep, disturbingly vivid dreams, and REM Sleep Disorder, characterized by acting out of dream content.
iv. Sensation disturbances

Very commonly reported sensation disturbances are impaired visual contrast sensitivity, spatial reasoning, colour discrimination, convergence insufficiency (characterized by double vision) and oculomotor control.

v. Autonomic disturbances

Oily skin and seborrhea dermatitis; urinary incontinence, typically in later disease progression; constipation and gastric dysmotility are more frequently noted autonomic disturbances in PD.

1.3 Epidemiology

Parkinson's disease affects approximately 1.5% of the population above the age group of 60 years and about 5% of the population above the age group of 80 years (29). PD is the second most common neurodegenerative disorder after Alzheimer's disease. The mean age at which PD is diagnosed is usually 65-70 years, but in many cases the onset of symptoms may precede clinical recognition by many years. PD is primarily a chronic, slowly progressive neurodegenerative disorder which takes about 15 – 20 years from the diagnosis of the disease until death. Clinical features of Parkinson's disease show bradykinesia (slow movements), resting tremors and muscular rigidity. Furthermore, patients may suffer from blurred vision, orthostatic hypertension, loss of smell, abnormal sleep patterns, nausea, constipation, bladder disturbances, fatigue, and approximately 40% show signs of depression (30). These symptoms result from the loss of dopamine and dopamine producing neurons in the nigrostriatal pathway of the basal ganglia (31). Clinical diagnosis is mainly depending upon clinical symptoms as brain imaging techniques do not always provide conclusive evidence for the condition (32). When parkinsonian symptoms are first identified, it is observed that, most of the patients have an 80% decrease in dopamine production in the putamen and a 60% loss of neurons in the substantia nigra pars compacta. The dopamine degeneration activity is specific to the nigrostriatal pathway; and neurons in the ventral segmental area of the midbrain that comprise the mesolimbic pathway are relatively spared (33). The function of the neuronal degeneration occurs naturally as a part of aging, but the degeneration that occurs during
Parkinson's disease is distinct and selective to the nigrostriatal pathway. Neuro
degeneration of PD is specific to the motor pathway. There is also more terminal loss
than cell body loss, which is normally not seen in the normal aging process. Such
observation also shows that striatal terminals are more involved and perhaps more
valuable than the cell bodies in the neural mechanism of the disease (34).

Incidence rate of PD is hampered by differences in methodology and reporting.
For example, some studies show crude incidence rates, others show incidence rates
adjusted to different standard populations. Most of the studies on PD done in Europe,
show incidence rates between 9 and 19 per 100,000 person-years (35). European studies
which mainly depend upon populations above 55 or 65 years shows nearly small
variation, total incidence rates between 410 and 529 per 100,000 person-years (36). In
North American studies show incidence rates ranging between 11 and 13 per 100,000
person-years (37). One recent North American study gives a report of incidence rate of
224 per 100,000 person-years in individuals 65 years or above (38). Asian studies give
incidence rates between 1.5 and 17 per 100,000 person-years (36). Another study in
Singapore reported an incidence rate of 32 per 100,000 person-years (39).

The incidence of PD is higher in men compared to woman. A meta-analysis
technique was used to study the incidence rate of 7 patient's by conducting door to door
survey which showed a male to female ratio of 1.49 (95% confidence interval, CI, 1.24-
1.95). Similarly, another meta-analysis conducted on 17 incidence studies of PD reported
a pooled age-adjusted male to female ratio of 1.46 (95% CI 1.24-1.72) which gives
significant heterogeneity between studies. All these results will indicate the male
preponderance of PD probably due to protective effects of estrogens, higher frequency of
intensity of occupational toxin exposure as well as minor head trauma in men, and
recessive susceptibility genes on the X chromosome (36).

Rochester Epidemiology Project is a huge data which suggests a lifetime risk
estimate of 2% in men and 1.3% in women. From age 40, the remaining lifetime risk was
1.7% overall. Incidence rate is higher in the Men, but the gender difference decreased
with increasing age, as men had higher mortality rates than women. Another study
conducted by Physicians Health Study reported higher lifetime risk among men (6.7%
after age 45) (31). These results conclude that probably the longevity of the study or over

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diagnosis may be another possible reason for higher life time risk among men compared to women (40).

1.4 Prevalence of Parkinson’s Disease

The overall Prevalence of Parkinson’s disease in door-to-door studies in elderly population (above 60 or 65 years) ranges from 154 to 167 per 100,000 (37). The survey done by the Parkinson Society revealed an overall prevalence between the range of 31 and 970 per 100,000. Some US and European studies show the PD prevalence rate among people of 65 years or older at 950 per 100,000 (38). Many European studies and country specific population structure data estimated that number of individuals above age of 50 with PD in the world at between 4.1 and 4.6 million in 2005. By year 2030 the number is projected to reach more than double between 8.7 to 9.3 million (41). The prevalence rate of PD is very low in Africa (42), Asia (43) and South America (44) compared to Europe. Other countries are also having low prevalence rate including Japan, China, Libya, Sardinia and Poland (45).

A group of Chinese scientists who examined 29,454 individuals (94% of the study population) reported a prevalence rate of 1,700 per 100,000 in individuals above 65 years (46). In Brazil prevalence rate of PD was found to be 3,300 per 100,000 in age group of above 65 years (47).

The prevalence rate of PD in Africa is very low due to shorter life expectancy compared to developed countries (42). The initial studies suggest that the prevalence rate of PD in Africa is very low compared with eastern and western countries, where life expectancy is lowest, where as prevalence rate in northern African countries (44) is very similar to those in developed countries.

Almost all epidemiological studies reveal that; prevalence rate of PD increases with age. However, some studies show decrease in prevalence rate in the oldest age group (above 80 or more) (37). This may be due to under diagnosis of PD because of co morbidity, non-response and unstable estimates due to small numbers in old age groups.

However many studies show that the prevalence rate is higher in men compared with women. Some studies also revealed that there is no gender difference (48).

The alarming rise in the prevalence of PD in India has been attributed to the demographic pattern, changing environment, as well as lifestyle (49) but some studies...
conducted in India reported that prevalence of PD in Indian population is lower compared with the western population (50-53). In Karnataka, the prevalence of PD is reported to be 7 per 100,000 (54). In the later studies, the prevalence of PD above 50 years of age was 134 per 100,000 (55).

1.5 Risk Factors in Parkinson’s disease

Metals

Chronic exposures to heavy metals like copper, iron, lead, manganese and mercury may contribute to development of neurological diseases, as these metals target the central nervous system. Manganese, iron, mercury and occasionally copper, occur in the form of fumes emitted in various occupational settings. Lead, copper and mercury these metals can spoil food and water sources (56). These are some of the major components of the neural mechanism that cause dopaminergic depletion in the basal ganglia (57). In another study, iron and copper present in the Substantia Nigra are supposed to interact with α-synuclein in-vitro in addition to causing oxidative stress through various enzymatic reactions (58). α-Synuclein aggregates in dopaminergic neurons contributing to their dysfunction. Finally, lead and mercury may disrupt dopaminergic transmission. Although the mechanism in which lead and mercury affect in dopamine function is not fully understood, the epidemiological studies give further evidence to support that exposure to these metals contributes to abnormal dopamine transmission (59).

Pesticides

Pesticides are found to be a causative agent of the Parkinson’s disease. Epidemiological studies suggest that Parkinson’s disease increases in individuals due to high exposure to pesticides through occupational or residential settings (60).

Fungicides

Fungicides are particularly used for crops to prevent molds and fungus. These fungicides contain metal or sulfur base but these may not produce severe toxic effects independently. There is one study which shows that some fungicides, such as Maneb can make the neurotoxic effects of other more potent pesticides like Paraquat (61).
Herbicides

Herbicides are one of the risk factor for Parkinson’s disease. Bipyridyl Paraquat is used globally because it has a structure similar to that of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and is one of the risk factor of the PD. It is a synthetic drug that gives Parkinsonian-like symptoms (62). Both epidemiological and animal studies have failed to prove a direct link between Paraquat exposure and the development of Parkinson’s disease (63).

Insecticides

Some research work has been done on neurotoxicity of insecticides especially Rotenone. Rotenone is an odourless, colorless, crystalline ketonic chemical compound used as a broad-spectrum insecticide and pesticide. It occurs naturally in the seeds and stems of several plants, More studies are required to prove that exposure to rotenone would damage dopaminergic neurons and cause the protein inclusions that resemble lewy bodies (64).

Organochlorines

Organochlorines are a family of highly chlorinated insecticides that were used in large quantities in the 1940s. A mixer of widespread usage and bioaccumulation in soil, water and human tissue results in high levels of exposure and deposition. In the 1970s, there was a large-scale ban on using organochlorines as agriculture products. For the past several years, global organizations have continued to ban usage of specific organochlorine products; however some part of the chemicals remain in the environment (65). In some parts of the world, acute exposures occur in occupational settings due to usage of organochlorines, while more commonly; chronic exposures occur from food consumption. Numerous reports and case control studies have shown that levels of organochlorines in the body are associated with higher incidence of Parkinson’s disease (66).

Dieldrin is a cyclodiene organochlorine which plays an important role in Parkinson’s disease. Despite its discontinued use, researchers identified dieldrin residues present in post-mortem brain samples of Parkinson’s patients (67). In animal models, dieldrin has been seen in striatal dopamine and increases dopamine transporter (DAT) levels as well as the vesicular monoamine transporter (VMAT2) which is responsible for...
packing dopamine prior to release (68). Further study gives, developmental exposure to dieldrin increased the neurotoxicity of MPTP when administered later in life. Dieldrin shows normal mitochondrial function, increases ROS and quinone levels and initiates apoptosis of dopamine neurons. Finally, effects caused by dieldrin appear to only impact the dopaminergic system (69). Such results would suggest that dieldrin varies the dopaminergic system and as a result of this it is likely to develop the Parkinson's disease.

Heptachlor is another type of cyclodiene pesticide which is used for both household and agricultural purposes. It will also target the dopaminergic system. Just like dieldrin, heptachlor was also banned for a number of years, however it bioaccumulates in the environment and as such the individuals will be continue to be exposed through the consumption of agricultural products. Animal studies have also shown that heptachlor plays an important role in development and increases VMAT2, DAT and TH levels. Although VMAT2 levels are elevated by the following exposure, heptachlor actually hinders VMAT2 transport (70).

These studies show that organochlorine pesticides cause severe toxicity to the dopaminergic system by increasing dopaminergic markers, such as TH, DAT and VMAT. Endosulfan is another organochlorine insecticide. However, unlike heptachlor and dieldrin, it is still used worldwide (71). Very little is known about endosulfan’s impact on the dopaminergic system but further research on its mechanism could clarify the role that endosulfon plays in the development of Parkinson’s disease.
1.6 Aims and Objectives

The objectives of the present study are

• To make an attempt to understand the epidemiology of Parkinson’s disease in North Karnataka.

• To understand the role of SNCA gene in Parkinson’s disease.

• To identify the mutations in SNCA gene in Parkinson’s disease in North Karnataka.

• To identify the novel mutations in SNCA gene for early diagnosis of Parkinson’s disease in North Karnataka.

• To generate some significant data regarding Molecular Biology of Parkinson’s disease in North Karnataka.