

A Pilot Study on Presence of Parkinson's Disease Risk Gene PARK7 in Population of West Bengal, India: A Preliminary Observation

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ABSTRACT

Introduction: The neurodegenerative Parkinson's Disease (PD) is associated with different risk genes along with some environmental factors. Till date, there are few identified genes which play a pivotal role in the early onset of PD among the general population.

Aim: To screen the presence of different risk genes of Parkinson's disease along with associated environmental factors among randomly selected 62 urban participants.

Materials and Methods: The present randomised control trial single blind pilot study was conducted in the Department of Biotechnology, Heritage Institute of Technology, Kolkata, West Bengal, India, from March 2021 to June 2021. The study participants were informed about the study and blood was collected from them. The genomic Deoxyribonucleic Acid (DNA) extraction was carried out following phenol chloroform extraction protocol and Polymerase Chain Reaction (PCR) was done with

specific primers of the risk genes namely, Parkinson Disease 1 (PARK1) Alpha-synuclein (α -synuclein), Parkinson Disease 2 (PARK2) (Parkin), Parkinson Disease Protein 7 (PARK7) or DJ-1 and PINK 1. Protein Deglycase-1 (DJ-1) and PTEN Induced Kinase 1 (PINK1). The statistical analysis of the findings was done using Statistical package for the Social Sciences (SPSS) software version 21.0.

Results: Out of 62 participants, 47 were males and 15 were females and the age distribution of the participants was in between 21 years to >60 years. After obtaining specific band in agarose gel electrophoresis, 35 positive cases of PARK7 gene and 1 positive case of PARK2 gene were identified.

Conclusion: There was a statistically significant association of different bacterial and viral infections with the PARK7 gene positive cases. PARK7 gene responsible for early onset of PD is significantly prevalent in the general population of West Bengal.

Keywords: Parkinson disease protein 7 gene, Parkinsonism, Polymerase chain reaction

INTRODUCTION

The condition of "Parkinsonism" is associated with several factors such as risk genes, varied clinical subtypes, and the supposed causative environmental factors [1]. In the year 1817, James Parkinson referred this clinical syndrome as "Essay on the shaking palsy", and later this was known as PD [2]. The disease PD is associated with the specific features such as tremor during rest, postural instability, rigidity and bradykinesia. Along with the above mentioned symptoms, the disease is associated with several other motor and non motor symptoms [2].

With the advancement of medical treatment facilities, and with increased life span of the overall population, age related disorders like PD are getting attention from the scientific community [2]. There is a lot of existing debate with regard to the association of PD with aetiological factors like lifestyle and environmental and other putative genes responsible for the condition [3]. It has been found that the disease is more frequent among the males than females (with ratio ranging between 1.3 to 2) [4]. However, the incidence rate is dependent upon varied other factors like smoking behaviour, intake of caffeine, consumption of postmenopausal hormones, exposure to heavy metal or pesticides, living in rural environment, those who have undergone traumatic head injury, having history of type 2 diabetes and melanoma, and many other factors [5-7].

Alpha-synuclein (SNCA) is considered to have a key role in the development of pathogenesis of PD, however, this particular mutant protein is an infrequent cause of PD. In this regard, other studies have reported that the duplication, triplication and quadruplication of the SNCA protein are associated with the pathogenesis of PD. Moreover, the polymorphic variant in the promoter region of SNCA results in the uprising sporadic cases of PD. The triplication of this particular gene is directly related with early onset of the disease and this is often referred as "gene dosage effect" [7,8].

Parkin is considered to be the very common autosomal recessive gene (PARK-2) associated protein responsible for PD. Nearly, 50% of the patients who are compound heterozygotes suffer from early onset of PD. Following autopsy, it can be observed that there is substantial loss of neurons within the substantia nigra pars compacta, however, there is well preserved dorsal tier with much less Lewy bodies [7].

The PARK7 or DJ-1 gene is also being referred to as oxidative stress sensor. The protein associated with this gene takes part in the development of oxidative stress related diseases such as cancer, type 2 diabetes, neurodegenerative diseases, and male infertility. Moreover, extensive researches have revealed that DJ-1 gene plays a role in immune and inflammatory disorders. This particular gene was originally being referred as oncogene and the putative gene for autosomal recessive early onset PD. The protein influences the activation of varied immune cells like mast cells, macrophages, T cells via Reactive Oxygen Species (ROS) dependent or independent pathways. The mutation Leucine166 Proline (L166P) in DJ-1 gene, is responsible for the familial occurrence of PD [9].

It is reported that loss of function of PINK 1 gene is the cause of early onset and recessive PD. The mitochondrial serine-threonine protein kinase is encoded by PINK 1 and it also stands beside PARK2 and DJ-1 gene those are responsible for early onset and recessive PD [10]. Moreover, interestingly some researchers have claimed that there is an indirect association in between bacterial or viral infection with the development of PD [11-14]. Researcher has shown an important association in between Hepatitis C Virus (HCV) infection and risk of PD development with the aid of epidemiological study and also with *Helicobacter pylori* infection [15,16]. Thus, in this present study, the prevalence of four genes discussed above namely PARK1, PARK2 and PARK7 and PINK 1 were screened among randomly selected 62 healthy volunteers.

MATERIALS AND METHODS

The present randomised control trial single blind pilot study was conducted in the Department of Biotechnology, Heritage Institute of Technology, Kolkata, West Bengal, India, from March 2021 to June 2021. The study was approved by the Institutional Ethical Committee (HIT/ IEC/2020/001/2021) and the entire study was conducted maintaining the ethical regulations as per the revised guidelines of Helsinki Declaration, 2013. The participants were informed in detail about the research investigation, about their participation and were asked to give their consent in the informed consent forms. Study was conducted with 62 adult participants selected randomly. The participants were randomly selected individuals of Heritage Institute of Technology, residents. An advertisement was mailed to all of the individuals of the Heritage Institute of Technology detailing about the significance of the research investigation and were asked to enroll for the research study, if they are willing to participate.

Inclusion and exclusion criteria: Urban residents who agreed to participate in the study after signing the informed consent following listening to the details and significance of the research investigation were included in the study. Patients who did not give consent to participate in the study were excluded from the study.

Study Procedure

The study participants were detailed about their rights to participation in a clinical investigation. They are randomly selected individuals of general population not patients of PD. Few of the study participants had history of Parkinsonism or other neurological disease which were also recorded. Family members were considered those who were in the blood relation with the study participants of the present research investigation. Predefined clinical details were recorded of each participant along with past history, and family history. The entire procedure was carried out in the presence of expert professionals. The blood collection was done by a trained phlebotomist for the adult participants.

The volunteers were asked to fill up detailed questionnaire with respect to their previous clinical symptoms, family history, and medicinal history, if any. A medical professional and Assistant Professor of Department of Biotechnology, took all the detailed demographical, medical and family history of all the participants, who finally agreed to participate in the present research investigation.

Blood sample collection: A 2 mL of the venous blood was collected from each participant in an Ethylenediaminetetraacetic Acid (EDTA) coated sterile blood vial. Before the collection of the blood, the participants stated their full name and the informed consent were matched. After collection the vials were marked with the patient code and initial name of the participants for the future follow-up and laboratory examination. The vials were then transferred in cooled pack so that there was no haemolysis. The blood was then centrifuged at 1200 rpm for five minutes for the separation of the plasma. Then the plasma was separated carefully into sterile 1.5 mL cryovials and stored at -80°C deep freezer for future experimental purpose. The cryo-vials were marked with the patient code for the future identification purpose.

Genomic DNA extraction: The genomic DNA extraction was carried out using the phenol chloroform extraction protocol [11]. The eluted DNA was dissolved in 60 µL of elution buffer and the quantification of the isolated DNA was done using A260/280 ratio with an Ultraviolet-visible spectroscopy (UV-Vis) spectrophotometer (Agilent, Malaysia). The absorbance for pure DNA was considered to be 1.8. Lower absorbance of any sample below 1.8 was considered to be contaminated with proteins and phenols.

PCR reactions and condition: After the extraction of genomic DNA, the conventional PCR (Bio-Rad, T-100, USA) was performed maintaining the following reaction conditions mentioned below in the

[Table/Fig-1]. The forward and reverse sequences of the selected primers are tabulated below in [Table/Fig-2]. The PCR products were run through 1.5% agarose gel at 80 mV for 30 minutes and then visualised under UV transilluminator to observe the bands. A 100 bp molecular marker was kept as positive control during the gel electrophoresis procedure.

S. No.	Temperature (°C)	Time (in minutes)
1.	95	3
2.	95	1
3.	60	1
4.	72	1
5.	Go to Step 2 (40 cycles)	
6.	72	5
7.	4	Infinite hold

[Table/Fig-1]: PCR reactions conditions.

S. No.	Genes	Forward primer sequence	Reverse primer sequence
1.	PINK 1 (Exon 1)	GCCCCAAGTTTGTGTGAC	GCCCCAAGTTTGTGTGAC
2.	PARK1	ACCAAACAGGGTGTGGCAGAAG	CTTGCTCTTTGGTCTTCTCAGCC
3.	PARK2	CCAGAGGAAAGTCACCTGCGAA	CTGAGGCTTCAAATA CGGCACTG
4.	PARK7	GTCCTACTGCTCTGTTGGCTCA	CCACACGATTCTCAG AGTAGGTG

[Table/Fig-2]: The forward and reverse primers of the selected genes are given in tabular format below.

STATISTICAL ANALYSIS

The statistical analysis of the findings was done using SPSS software version 21.0. The correlation and association study was done and the result was considered significant at p-value<0.05.

RESULTS

The age distribution of the participants was in between 21 years to >60 years [Table/Fig-3]. It has been observed that among 47 (75.80%) male volunteers, 25 (24.19%) of them were positive for PARK7 gene and among them only one volunteer was also positive for PARK2 gene [Table/Fig-4]. Among the 15 female volunteers, 10 cases were found to be positive [Table/Fig-4]. In the [Table/Fig-5], the age-wise distribution of participants and the number of positive cases of PARK7 are mentioned. The agarose gel electrophoresis image [Table/Fig-6] depicted the positive sample of PARK7 or DJ-1 gene of 24 kb containing eight exons and the protein coded by this gene comprises of 189 amino acids [12]. There was significant association obtained in between different communicable diseases like chicken pox, typhoid, malaria, mumps, jaundice and measles with the PARK7 gene as obtained from their clinical history (p-value=0.0048) [Table/Fig-7]. Similarly significant association was also obtained with the any injuries/ surgeries and different diseases associated with the family members of the volunteers such as diabetes, hypertension, cardiovascular disease, cancer, neurological disorders, urological disorders, asthma and arthritis [Table/Fig-8,9].

Variables		n
Age (in years)	21-30	2
	31-40	12
	41-50	26
	51-60	16
	≥60	6
Occupation	Service at private organisation (other than teaching)	52
	Teaching faculties at schools and colleges	10

Religion	Hindu	56
	Muslim	1
	Christian	3
	Buddhist	2
Marital status	Single	4
	Married	56
	Widow/ Widower	1
Family history of PD (Yes/No)	21-30 years	No
	31-40 years	No
	41-50 years	Yes
	51-60 years	No
	≥60 years	No
Relation with PD in family	210016: Father	
	210021: Self	

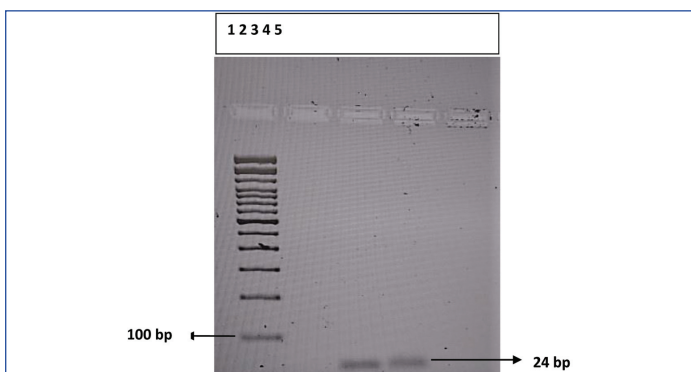
[Table/Fig-3]: Details of the study participants and their family members about their demographical details. The code 210016 and 210021 denotes volunteer ID.

PARK1 gene positive cases (n)	PARK2 gene positive cases (n)	PARK7 gene positive cases (n)	PINK 1 gene positive cases (n)
0	1	35	0

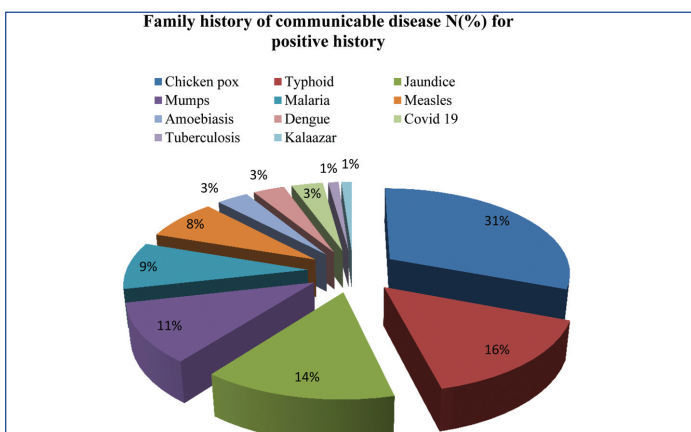
[Table/Fig-4]: Depicting the positive cases of four selected genes.

S. No.	Age distribution (years)	Number of participants (n)	PARK7 positive cases (n)
1	21-30	2	1
2	31-40	12	7
3	41-50	26	16
4	51-60	16	8
5	60 and above	6	3

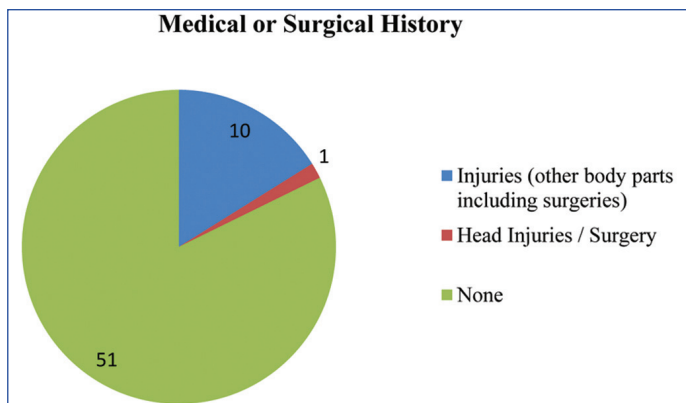
[Table/Fig-5]: Showing the age-wise distribution of participants and their association with positive cases of PARK7 gene.



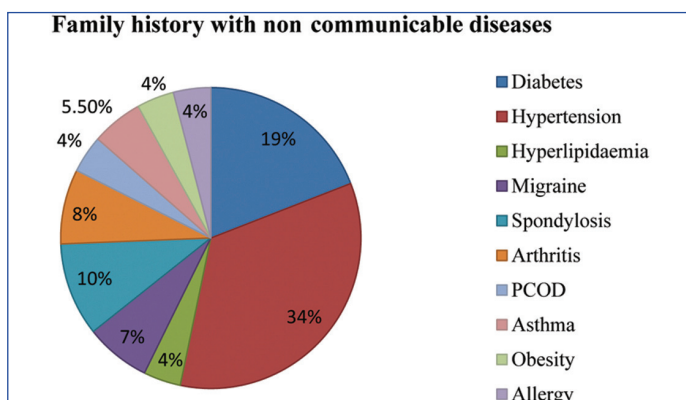
[Table/Fig-6]: Agarose gel electrophoresis of PARK7 gene positive and negative PCR products. Lane 1-100 bp molecular marker, Lane 2 and 5 negative sample; Lane 3 and 4 positive samples.



[Table/Fig-7]: Details of the study participants and their family members about their history of communicable diseases (p-value=0.0048).



[Table/Fig-8]: Details of the study participants about their previous medical history, and family history.



[Table/Fig-9]: Details of the study participants and their family members about their history of non-communicable diseases.

DISCUSSION

Literature studies have revealed that there is an association in between age and onset of PD among patients depending on mutations observed among DJ-1 or PARK7 and PINK 1 genes [15]. Researchers have reported about the onset of the disease before the age of 40 years. The symptoms of the disease such as bradykinesia, postural tremor, failure of postural reflexes, along with psychological symptoms appeared among the patients; however, the progression of the disease was found to be dawdling [15]. Similarly, other researchers also stated that there is strong association between PD and age of the study population [17]. It was reported that the frequency was 11.8% for people who are less than equal to 75 years, for age range in between 75-84 years, the frequency was within 29.1% and it was 43.7% for those above 85 years. In the pilot study, authors have found there were seven PARK7 (58.33%) gene positive cases among 12 persons within the age range of 31-40 years, among 26 participants, there were 16 positive cases (61.53%) for the age range within 41 to 50 years, 8 (50%) cases were positive among 16 participants within the age range of 51-60 years, and 3 (50%) cases were positive among six participants who are above 60 years [17]. With regard to the association of PD with gender, researchers have already reported that PD is found to be 1.5 times more common among males when compared to females [18]. They also stated that pitiable performance of cognitive variables such as processing speed and executive function was found to be more among males than females. The present study found that among the females the preclinical symptomatic manifestations are delayed and develops more of a benign PD tremor dominant TD (Tandem Duplicato) phenotype [18]. However, in the current study population comprised of 47 males and 15 females and among them 25 (54.34%) and 10 (66.66%) cases were positive for PARK7 gene, respectively. The difference in observation is due to the unequal division among the male and female number of participants due to which the positive cases are getting overhyped.

Manifestations of PD can be both due to genetic and environmental factors [13]. Some researchers have claimed that there is an indirect association in between bacterial or viral infection with the development of PD [11,12]. One study has shown an important association in between Hepatitis C Virus (HCV) infection and risk of PD development with the aid of epidemiological study and also with *Helicobacter pylori* infection [15]. The study also showed association of PD risk due to the infection and its response to levodopa. The explanation behind the association as provided by scientist is that infectious agents such as viruses and certain bacteria have neurotropic effects such as specifically susceptibility of the substantia nigra and also the encouragement of aggregation of Alpha-synuclein (α -SNCA) [15]. In the present study, also there was a statistically significant association of different bacterial and viral infections such as chicken pox, typhoid, malaria, mumps, jaundice and measles with the PARK7 gene positive cases (p-value=0.0048). The disease manifestations is considered to be due to the complicated interplay of variable factors (both environmental and genetic) that culminates to the development of PD. Certain environmental factors that have been highly inversely associated with the development of PD are cigarette smoking, exposure to pesticides, intake of caffeine, strenuous physical activity, and plasma urate, physical and emotional trauma, and also dietary components [14,16-19]. In 1918, postencephalitic PD has been reported following influenza pandemic and the disease has been recognised as encephalitis lethargica [15]. Therefore, viruses which are effectively neurotropic might be responsible for PD. Moreover, the "multiple microbe" hypothesis also claimed that risk of developing PD is more among the population who have suffered disease due to infection of the following agents such as Herpes simplex virus-1 (HSV-1), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), *Chlamydia pneumoniae* (*C.pneumoniae*), *Borrelia burgdorferi* (*B.burgdorferi*) and *Helicobacter pylori* (*H.pylori*) in comparison to healthy controls [15]. In the present study, there was a significant association in between various non communicable diseases that the family members of the participants have suffered and the positive cases of PARK7 gene. In this respect several authors have varied opinions where there is inverse association of PD and different forms of cancer cases. However, the association is direct between positive PD cases and different cardiovascular problems such as myocardial infarction, Chronic Heart Failure (CHF), ischemic stroke and several other symptoms [20].

Limitation(s)

As it was a pilot study, authors did not include all the relevant points, such as smoking behaviour, intake of caffeine, consumption of postmenopausal hormones, exposure to heavy metal or pesticides, living in rural environment, in the present research investigation.

CONCLUSION(S)

The gene PARK7, responsible for early onset of PD was significantly prevalent in the general population of West Bengal, India. Though

many factors were considered in details, within this pilot study, however all the mentioned points within the limitations of the study will be considered in the final study with a larger sample size.

Authors contribution: Author Debasmita Chatterjee has carried out the entire experimental process and written the manuscript. Author Krishnendu Paira and Debasmita Chatterjee have obtained the demographical and clinical details of each participant. Author Banhishikha Singh has also participated in the experimental analysis. Author Satadal Das has planned up the entire experiment, analysed the experimental findings and checked the final manuscript.

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