Absence of SNCA polymorphisms in Pakistani Parkinson's disease patients

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Abstract

Objective: To elucidate the genetic risk and role of alpha-synuclein gene in the pathogenesis of Parkinson's disease in Pakistani population.

Methods: This case-control study was conducted at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad from May 2013 to May 2016, and comprised patients with Parkinson's disease and their ethnically-matched healthy controls. Allele-specific polymerase chain reaction was used for screening of three pathogenic single nucleotide polymorphisms in alpha-synuclein gene. Moreover, 20% samples were randomly selected for bidirectional Sanger sequencing to confirm the results. SPSS 13 was used for data analysis.

Results: Of the 374 participants, 174(46.5%) were patients and 200(53.5%) were controls. The mean age for the onset of the disease was 55±13 years. No polymorphism was observed for rs104893875(G>A), rs104893877(G>A) and rs104893878(C>G) in alpha-synuclein gene in samples of patients and controls.

Conclusion: Alpha-synuclein gene mutations might not be relevant to all the populations in causing Parkinson's disease.

Keywords: Parkinson's disease, α-Synuclein gene, Pakistani population, Allele-specific PCR, SNCA. (JPMA 67: 1512; 2017)

Introduction

Parkinson’s disease (PD) is the second-most common age-related neurodegenerative disease.1 PD is clinically characterised by resting tremor, bradykinesia, postural instability and rigidity. The prevalence of PD is approximately 2% in the population of over 65 years age.2 The disease is usually considered sporadic with onset in old age and the aetiology is incompletely understood. PD is a complex disorder involving multiple genetic and environmental factors.3

The triggering factor in the pathogenesis includes the progressive loss of dopaminergic neurons of substantia nigra pars compacta. Typical motor symptoms initiate when the neuronal cell loss reaches 80% or above.4 The hallmark of PD is the post-mortem Lewy bodies identified in brain autopsy samples. Lewy bodies appear in intra-cytoplasmic inclusions with the dense eosinophilic core surrounded by clear areas after haematoxylin and eosin staining. Lewy bodies are composed of α-synuclein protein and fibrils.5 Nuytemans et al. and Yonova-Doing et al. reported that genetic and molecular profiling have identified more than 500 distinct mutations in five genes associated with PD; in alpha-synuclein (SNCA), parkin (PARK2), phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1), DJ-1 (PARK7), and leucine-rich repeat kinase 2 (LRRK2).6,7 Small insertions, deletions and frame shift mutations account for approximately 82% while the copy number variations are 18%.6

Genetically, SNCA mutations including point mutations and copy number variations are known to cause familial PD, further supporting the assumption that SNCA plays a crucial role in PD pathogenesis. The most common and well-studied polymorphisms in SNCA gene in context of PD includes rs104893875(G>A), rs104893877(G>A) and rs104893878(G>C).

Moreover, rs104893875(G>A) was first linked in a multi-generation Spanish family. Post-mortem staining of the brain slices showed atrophy of the substantia nigra.8 Functional studies have shown that rs104893875(G>A) polymorphism increases filaments assembly and aggregation of the alpha-synuclein protein.9,10 Similarly, rs104893877(G>A) mutation was first reported in Swedish family. Rs104893877(G>A) causes encephalopathy with cortical involvement and PD.11 An abundance of alpha-synuclein immunoreactive Lewy-neurites were found in the brainstem pigmented nuclei, hippocampus, and temporal neocortex in the patients harbouring rs104893877(G>A) polymorphism.12 The same
polymorphism was also found in small Korean family confirming the evidence of pathogenesis.\textsuperscript{13} rs104893878(G>C) was first reported in a family suggestive of autosomal dominant Parkinson's disease. The positron emission tomography (PET) scan of the family harbouring the rs104893878(G>C) mutation showed decreased fluorodopa (F-DOPA) uptake into the caudate nucleus and putamen. Hypo-metabolism was also found in frontal, parietal and left temporal cortex. Clinical re-evaluation confirms the memory loss and decrease in the intelligence.\textsuperscript{14} Post-mortem examination of patients' brain slices showed lewy bodies and neuro-degeneration.\textsuperscript{15} These studies point towards the importance of SNCA polymorphisms that could be a good candidate for molecular diagnosis of PD.

The current study was designed to find out possible association of SNCA polymorphisms in PD patients and spectrum of these polymorphisms.

Patients and Methods
This case-control study was conducted at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad from May 2013 to May 2016, and comprised PD patients and healthy controls. All the patients were clinically diagnosed by a neurologist at Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan. The controls were similar to patients according to their socio-economic background (e.g., rurality and occupational structure) and were recruited during the same period. The total sample size was enough to detect odds ratio of 7 with 80% power. The study was performed in accordance with the Declaration of Helsinki and was approved by the research ethics committee of the Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan. Written informed consent was obtained from all subjects. The controls were matched with the cases according to age, ethnicity, gender and lifestyle. All PD patients were subjected to a detailed interview designed to obtain demographic and clinical information. The Unified Parkinson's Disease Rating Scale (UPDRS) score was recorded for most patients. The tremor dominant (TD) and postural instability/gait difficulty (PIGD) phenotypes of Parkinson's patients were identified as described previously.\textsuperscript{16} Briefly, a ratio was obtained by dividing the sum of UPDRS "tremor items" 16, 20 and 21 by the sum of "postural instability and gait difficulty items" 13-15, 29 and 30. The cut-off scores of $\geq$1.5 and $\leq$1.0 were used for TD or PIGD phenotypes, respectively.

A venous blood sample of 5ml in acid-citrate-dextrose vacutainers (BD) were collected from all the subjects and samples were stored at 4-8°C until deoxyribonucleic acid (DNA) extraction. The genomic DNA was extracted from the peripheral blood leukocytes by standard phenol-chloroform method.\textsuperscript{17} All the DNA samples were stored at -20°C.

The DNA sequence of SNCA gene(gene ID 6622;
Human Genome Organisation Gene Nomenclature Committee (HGNC) number 11138; Online Mendelian Inheritance in Man (OMIM) number 163890) was retrieved from the National Centre for Biotechnology Information (NCBI) and primers for, rs104893875(G>A), rs104893877(G>A) and rs104893878(G>C) and were designed using online program Primer3. Two pairs of primers were designed for each single nucleotide polymorphism (SNP): one for normal allele and the other for mutant allele.

Allele-specific polymerase chain reaction (PCR) was carried out in 20µl reaction volume including 100ng genomic DNA, 1.5mM magnesium chloride (MgCl2), 1U of TaqDNA polymerase and 200µM each of forward and reverse primers. PCR was performed with 95°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, annealing at 64°C for 30 seconds and extension at 72°C for 60 seconds, and finally an extension at 72°C for 5 minutes. Subsequently, the amplicons were run on pre-stained 2% agarose gel electrophoresis along with 100bp DNA ladder. The gel was observed under ultraviolet (UV) transilluminator and images were captured. Genotypes were assigned on the basis of the presence or absence of allele-specific band. The reproducibility of the genotyping methods was confirmed with bidirectional Sanger sequencing performed on randomly picked samples (20%) using primers flanking the regions of interest. Sequences were aligned to observe any change.

The sample size and study power was calculated online (http://sampsize.sourceforge.net/iface/s3.html). SPSS 13 was used for the statistical analysis.

**Results**

Of the 374 participants, 174(46.5%) were patients and 200(53.5%) were controls. The mean age for PD onset was 55±13 years and the mean total UPDRS score was 87.8±32.5. Also, 29(16.7%) patients had tremor-dominant PD.

All the participants of the study were found homozygous for major allele for rs104893875(G>A), rs104893877(G>A) and rs104893878(G>C). There was no individual with minor allele (Figure).

**Discussion**

Alpha-synuclein is a protein which is highly expressed in the brain. The postulated function of the protein is...
vesicle formation for the storage and transportation of dopamine. Dopamine is transported from pre-synaptic to post synaptic neuron, and is required for the smooth and coordinated movement of the body. Mutated α-synuclein is incapable of making the vesicle for the transportation of dopamine and thus forms aggregates which is the hallmark of the pathology for PD.\(^1\)

Genetic alterations which disrupt the normal function of the α-synuclein protein are the underlying cause of Parkinson’s disease. Studies have been conducted to find the role of SNCA mutations in different populations and direct segregation of the disease mutation in families.\(^{11,13,20}\) To elucidate the role SNCA polymorphism(s) in Pakistani population in the current study, all the patients and controls were screened for the mentioned mutations, however, we did not find any polymorphism. The absence of mutations in SNCA gene was also reported previously in Indian population.\(^21\) The absence of SNCA polymorphisms in Indian and Pakistani population could be due to the geographical and ethnic distribution. Studies from the United States,\(^22\) United Kingdom\(^23\) and Russia\(^24\) also reported that SNCA mutations are not the cause of PD. In a Greek population, the absence of mutation in SNCA suggests no role of this gene in PD.\(^{25}\) Lack of SNCA mutation was also reported in a study from European population.\(^26\) In a study on Chinese Han population, the absence of SNCA polymorphism has also been reported.\(^27\)

The absence of SNCA polymorphism in Indian, Chinese, Russian and Pakistani population suggests that Asian population do not exhibit these polymorphisms. The presence, absence and geographical or ethnic distribution of the polymorphisms are not limited to SNCA only. But they have also been reported in the other genes. Li et al. reported the geographic and ethnic distribution of two polymorphisms (D/N394 and L/I272) of the parkin gene in sporadic Parkinson’s disease.\(^28\) Similarly, Binia et al. and Wang et al. reported the ethnic and geographical distribution of the methylene tetrahydrofolatereductase (MTHFR) polymorphism.\(^29,30\) Such geographical distribution of SNPs helps to identify the ancestry.\(^31\)

**Conclusion**

The SNCA mutation was absent in the PD patients. Thus SNCA polymorphisms have very limited or no role in causing PD.

**Disclaimer:** The study is part of a PhD thesis.

**Conflict of Interest:** None.

**Source of Funding:** None.

**References**