ORIGINAL ARTICLE

# PITX3 GENE POLYMORPHISM AND ITS ASSOCIATION WITH PARKINSON'S DISEASE

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**Background:** PITX3 is a paired-like homeodomain transcription factor-3, a gene which controls the development of ocular lens and dopaminergic neurons in the midbrain. Previously three major single nucleotide polymorphisms (SNPs) in PITX3 gene have been reported, i.e., rs3758549, rs2281983 and rs4919621. Our goal of study was to determine whether SNP rs4919621 is a risk factor for onset of Parkinson's disease (PD) in Pakistani population, specifically in KPK region. **Method:** This multicentered study includes 321 samples (91 cases of Parkinson's disease and 230 healthy controls) taken from Hayatabad Medical complex, Lady Reading Hospital and other clinics in Peshawar. Samples were also received from Institute of Biomedical Engineering Islamabad. DNA was extracted using modified salting out protocol. PITX3 polymorphism was genotyped using amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method. Chi-square was done to determine the association between PITX3 genotypes and PD. Parkinson's disease risk was calculated using odds ratio at 95% confidence interval. Hardy-Weinberg equilibrium (HWE) was used to determine genotype distribution in samples. **Result:** Our study showed significant association between PITX3 gene polymorphism (rs4919621) ($p$=0.0138) and PD with odds ratio of 0.26. **Conclusion:** PITX3 SNP rs4919621 is significantly associated with PD but not a risk factor for development of disease. Studies on a larger scale must be conducted to elaborate the risk of PD in patients with PITX3 gene polymorphism.

**Keywords:** Parkinson's disease, PITX3, risk factor, genetic polymorphism, rs4919621

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## INTRODUCTION

Parkinson's disease (PD) is the 2nd most prevalent neurological disease after Alzheimer's disease that causes movement abnormalities and mostly affects white people than the blacks, i.e., its occurrence is higher in America, Europe and Australia than the Asian countries.[1,2] The global load of all neurological disorders is about 6.5% of total diseases and it is thought that the prevalence of these neurological disorders ranges from 4–5% in developing countries like Pakistan.[3] The presence of disease among different age fellows varies and is not uniform. About 1% of the population suffers from PD at age of 65 years, but it tends to increase by 5% at the age of 85 years. The average age for the onset of disease is 70 years.[4]

Paired-like homeodomain transcription factor 3 (PITX3) is a gene located at q24–25 region of human chromosome 10 and contains 4 exons. It encodes a protein that take part in development of ocular lens and dopaminergic neurons in midbrain region.[5,6] The expression of PITX3 in mesencephalon dopaminergic neuron (MesDA) is sustained throughout the life in humans. Reduction in MesDA neurons was observed in PITX3 knockout mice with hypokinetic activity by Munckhof *et al*[7] which ultimately shows the importance of PITX3 gene expression for survival of MesDA neurons. Extra-neural expression of PITX3 was shown in development of eye lens.[8]

The genetic mutations in PITX3 gene results in cataracts formation and the loss of dopaminergic neurons in mesencephalon leading to onset of motor disorders.[9,10] Mutation in PITX3 has been detected in two families with inherited cataract.[11] Similar abnormality was detected in eye lens from mice with two deletion in PITX3 gene.[12] There are three single nucleotide polymorphisms (SNPs) identified in different regions of PITX3 gene.[13] In a meta-analysis study analysing the prevalence of major SNPs, i.e., rs3758549, rs2281983 and rs4919621. The result showed that there is no notable genetic difference between the cases and control in Caucasians and Chinese groups. Hence it was indicated that these SNPs are not major risk factors for onset of Parkinson's disease.[14] Le *et al*[15] in a study showed that the rs2281983 and rs4919621 are major determinants of pathology of PD, particularly in early onset of the disease.

Undeniably, adequate research has not been performed to determine the link between PITX3 gene polymorphism and PD in Pakistan. The linkage disequilibrium (LD) pattern between the PITX3 gene polymorphisms has not been studied with respect to PD risk. In conclusion, no such studies have been conducted in Pakistani population. As per literature review, the two

SNPs were not significantly associated with onset of PD, i.e., rs3758549 and rs2281983. The goal of our study was to determine whether SNP rs4919621 is a risk factor for onset of PD in Pakistani population, specifically in KPK region. We analyzed only one SNP, i.e., rs4919621 in 91 Parkinson's disease patients and 230 controls.

## SUBJECTS AND METHOD

The study was approved by the Ethical Committee of KMU (Reference number: DIR/KMU-EB/PG/000179). A total of 321 study samples comprising of 91 patients (38 females and 53 males) and 230 control (106 females and 124 males) were analysed in this study, who had no other neurological disease. The number of cases and controls were different due to low prevalence of PD in our study population.

The blood samples were obtained from the patients from Outpatient Department, or were admitted in Hayatabad Medical Complex, Lady Reading Hospital, and other healthcare facilities in Peshawar. Samples were also obtained from Institute of Biomedical and Genetic Engineering (IBGE), Islamabad and experimental work was carried-out at Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. Informed consent for participation in the study was obtained from all subjects. The patients who were willing to be a part of our research project were included in the study. Through venipubcture, 3 ml blood was withdrawn and transferred to EDTA coated vacutainers and kept at -4 °C.

DNA was extracted from whole blood by using modified salting out protocol[16] and was kept at -20 °C. For analysis of specific PITX3 SNP (rs4919621), specific primers were designed. The amplification products were analysed by loading 10 µl of PCR product on 1.5% agarose gel along with 1 µl of 6X loading dye and 1 kb DNA ladder was used for size discrimination. The results were then visualized under ultraviolet (UV) trans-illuminator (Figure-1).

Statistical analysis was done using SPSS-20. Chi-square test was performed for finding association between alleles and genotypes. Allele and genotype frequencies were assessed under Hardy-Weinberg equilibrium (HWE). Odds ratio (ORs) was calculated with 95% confidence interval (CI).

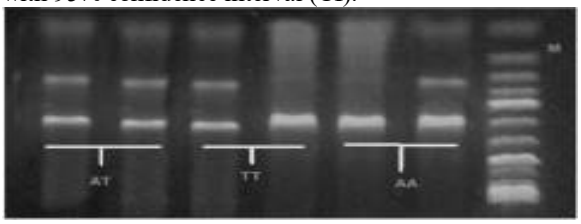


**Figure-1: PCR amplification of PITX3 gene.**
(Right to Left) Lane 1: DNA gene ruler ladder, Lane 2: AA, Lane 3: TT, Lane 4: AT. PCR product size of 273 bp for Allele specific, 754 bp for Control

## RESULTS

The association study between PITX3 SNP rs4919621 and PD was conducted in 91 PD cases and 230 healthy controls. Chi-square test showed significant association between the SNP and PD ($p$=0.0138). Odds ratio (OR) between the cases and controls to check whether this significant association is a risk factor for the onset of disease in patients, was calculated. The calculated value of odds ratio (OR) was 0.26 (Table-1). The genotype distribution of polymorphism was not consistent with Hardy-Weinberg equilibrium (Table-2).

**Table-1: Analysis of association of PITX3 polymorphism (rs4919621) with PD**

| | Sample type | n[b] | Genotype Frequency | | | | | Allele Frequency |
|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | $p$[c] | OR (95% CI)[d] | T[e] |
| | | | AA | A/T | TT | | | |
| SNP[a] | Cases | 91 | 9 | 55 | 27 | 0.0138 | 0.26 (0.34–0.18) | 0.901 |
| | Controls | 230 | 73 | 82 | 75 | | | 0.682 |

a: SNP name for genotype in cases and controls. b: Number of valid subjects who were successfully genotyped for SNP. c: Analysis performed by a 2×2 table for SNP using major heterozygotes *vs* others in cases and controls. d: Reference groups designated with OR of 1.00. e: Specific allele frequencies in cases and controls.

**Table-2: Genotypes distribution in Hardy Weinberg equilibrium**

| Genotype[a] | Observed value[b] | Predictive value[c] | $\chi^{2d}$ | $p^e$ |
|---|---|---|---|---|
| **CASES** | | | | |
| **AA** | 9 | 14.6 | 6.05 | 0.0138 |
| **TT** | 27 | 43.7 | | |
| **AT** | 55 | 32.6 | | |
| **CONTROLS** | | | | |
| **AA** | 73 | 56.5 | 18.93 | <0.05 |
| **TT** | 75 | 58.5 | | |
| **AT** | 82 | 115 | | |

a: Allele specific homozygotes and heterozygotes, b: Experimental value for allele specific PCR amplification, c: Predictive value by HWE, d: Fischer Exact's test value, e: Analysis performed by a 2×2 table for SNP using major heterozygotes *vs* others in cases and controls.

## DISCUSSION

The PITX3 gene is located between D10S1239 and D10S1237 of chromosome 10q which is thought to be associated with PD.[15,17–19] The PITX3 knock-out mice were unable to develop DA neurons in mesencephalon on brain.[20] These DA neurons have important role in onset of movement disorders such as PD. Hence, normal physiology of these neurons is necessary for synchronized body movements.

This case-control study was designed to analyse one of the three previously investigated PITX3 SNPs, i.e., rs4919621 to investigate the frequency of PITX3 gene polymorphism in PD patients. Due to less prevalence of PD, we took more healthy controls than the diagnosed patients. Our results also confirmed the significant association between rs4919621 and PD ($p$=0.0138) as described by Bergman *et al*[18]. Although, it was previously found that none of the known SNPs in

PITX3 gene cause change in amino acid sequence in transcript protein, it can be suggested that PITX3 SNPs may result in change in function of the protein which influence the pathogenesis of PD.[21]

There was a statistically significant association between PITX3 polymorphism (rs4919621) and Parkinson's disease in our study, but it is not a risk factor. One reason for this can be that PITX3 polymorphism rs4919621 is related to early onset of the disease rather than the late onset of the symptoms[18]. However, these results were not replicated in study performed by Yuan *et al*[22] on Chinese population which may be due to genetic or environmental factors. Other factors contributing to onset of PD include diet, lifestyle and exposure to hazardous chemical substances.

## CONCLUSION

PITX3 polymorphism (rs4919621) showed significant association with the PD and can be speculated as one of the factors contributing to onset and development of PD. However, it is not a risk factor for development of disease in susceptible patients.

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**Contribution of Authors:**

**MA:** Concept, Study design, Manuscript writing and review
**AM:** Data collection and analysis, Review
**SH:** DNA Extraction
**SI:** Data analysis, Results compilation
**MIS:** PCR, Script writing, Revision
**HM:** Data collection

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