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Screening of Mutation in *parkin* gene – *exon* 3 for diagnosis of Parkinson's Disease

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The present study focuses to screen the mutation in *parkin* gene (exon3) of Parkinson's diseased patients by collecting blood samples from 16 early onset Parkinson's disease patients in the age group of below 45 years. To detect these mutations, we performed an effective technique based on the real-time TaqMan PCR system. The amplified product was subjected to sequence analysis for confirming mutation in *Parkin* gene (exon3). The chromatogram was collected and subjected to sequence alignment using BLAST software. The sequenced exon 3 was visualized for the presence of Mutation. In this study, we have not identified any mutation in exon 3 and conclude that there are possibilities for the involvement of other exons in induction of this disorder to become the basis for a diagnostic test.

Key words: Parkinson's disease, DNA, gene, *parkin*, exon 3 and mutation

Parkinson disease (PD) is one among the highest neurodegenerative disease following the Alzheimer, with approximately six million cases have been reported worldwide[1]. The PD is characterized by neurodegenerative movement disorder, with a syndrome of tremor, muscular rigidity, slowing of physical movement (bradykinesia), and loss of physical movement (akinesia)[2.] The present scenario in PD research vividly shows that there is increasing evidence of genetic factors contributing to sporadic PD. The death of dopaminergic neurons in the substantia nigra pars compacta is the major causative phenomenon of PD.

In most patients with Parkinson's disease, treatment is effective in the early stage of diagnosis, but not all symptoms cure and the expediency of treatment is eventually vulnerable by the emergence of drug-resistant symptoms, drug-induced maladies, or both[3]. In recent years, there are increasing numbers of genetic disorder found to be associated with familial PD. At present there are more than 18 genes have been identified to be associated with the monogenic types of parkinsonism and related disorders. These specific genes, code for an autosomal recessive (AR) mode of inheritance, such as *parkin* (*PARK2*), *PINK1* (*PARK6*), *DJ–1* (*PARK7*), *ATP13A2* (*PARK9*), *PLA2G6* (*PARK14*) and *FBXO7* (*PARK15*), are found in patients with not only familial PD, but also sporadic PD[4,5,6,7,8]. *PARK2* (*Parkin*) gene mutations were first identified in autosomal recessive juvenile with the onset of Parkinsonism (ARJPD)[9]. It is located in the chromosomal location 6q25.2-q27[10]. Mutations in the loci of Parkin gene result in the loss of Parkin function, slow down the

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destruction of the defective proteins causing them to accumulate in the cell, and lead to the nigral neuronal degeneration[11]. Parkin is one of the largest human genes comprising of size 1.38 Mbp. It consists of 12 coding exons separated by large intronic regions. The parkin gene translates to about 465-amino acid protein, corresponding to a ubiquitin-like domain at the N terminus and a RING (Really Interesting New Gene) loci possessing three RING finger motifs (RING0, 1, and 2). RING 1 and 2 are separated by a sequence without any recognizable domain structure named IBR (in between-RING)[12].

The present study focuses specifically on the screening of mutation in the *exon* 3 of *parkin* gene for the early diagnosis of Parkinson's disease with special reference to young age group below 45 years.

MATERIALS AND METHODS

The Peripheral blood (3 mL) was collected from clinically diagnosed 16 PD patientsin the age group of below 45 years. Genomic DNA was isolated from patients and controls using standard protocols. The exon 3 of the parkin gene was amplified by PCR using the below mentionedprimer (Table 1). All reaction was performed in 10-µl reaction mixtures, containing 10X PCR buffer, 15 mM $Mgcl_2$, 10 mM DNTPs, 5 pmol of each forward and reverse primer and 0.01 µl of Taq DNA polymerase. The initial denaturation at 95º C for 5 min was followed by 30 cycles of denaturation at 95º C for 40 s, 57º C for 45 s, 72º C for 45 s, and a final extension at 72º C for 5 min. The PCR products were electrophoresed on 2% agarose gel and visualized with ethidium bromide. The PCR amplified exon 3 of parkin gene was purified using the axygen PCR purification kit. Purified PCR product were sequenced an automated sequencer according to the manufactures' recommendations to detect mutations.

RESULTS AND DISCUSSION

In the 16 patients analyzed The amplified PCR product of Parkin gene (exon 3) was analyzed for the unknown mutation. The documented gel exhibits band size 241bp (Fig .1).

The PCR amplicons of Parkin exon 3 was analyzed for the unknown mutation by sequencing the respective amplicons. The mutation was found in the control. The nucleotide sequence was aligned using blast software and chromatogram

Table 1. Primer Sequences for RT- PCR

Gene	Sequence
PARK2 (exon 3)	Forward 5' AATTGTGACCTGGATCAG 3' Reverse 5'CTGGACTTCCAGCTGGTGGTGAG 3'

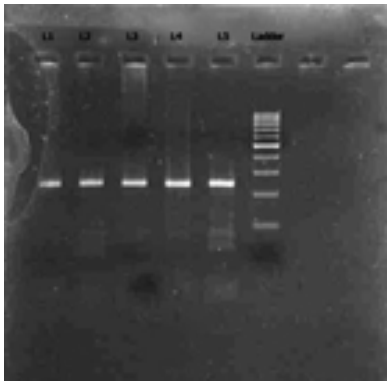

Fig.1. Documented gel of Parkin gene (exon 3) [L1-L5 Samples; L6-DNA Ladder]

was collected on the parkin gene sequence (Fig.2).Sequencing confirmed that the RT-PCR products were the parkin gene (Fig.3).

In this study, we have analyzed 16 early onset Parkinson's disease patients for exon 3 mutation. In common, more than 80 % and above mutations were present throughout the parkin gene and we have directly sequenced the exon 3 for the presence of mutation. In this study, we have not identified any mutations in this exon 3. Hence the possibilities of the involvement of the other exons, a complete examination of the whole gene may be followed. We hereby conclude that there are possibilities for the involvement of other exons in

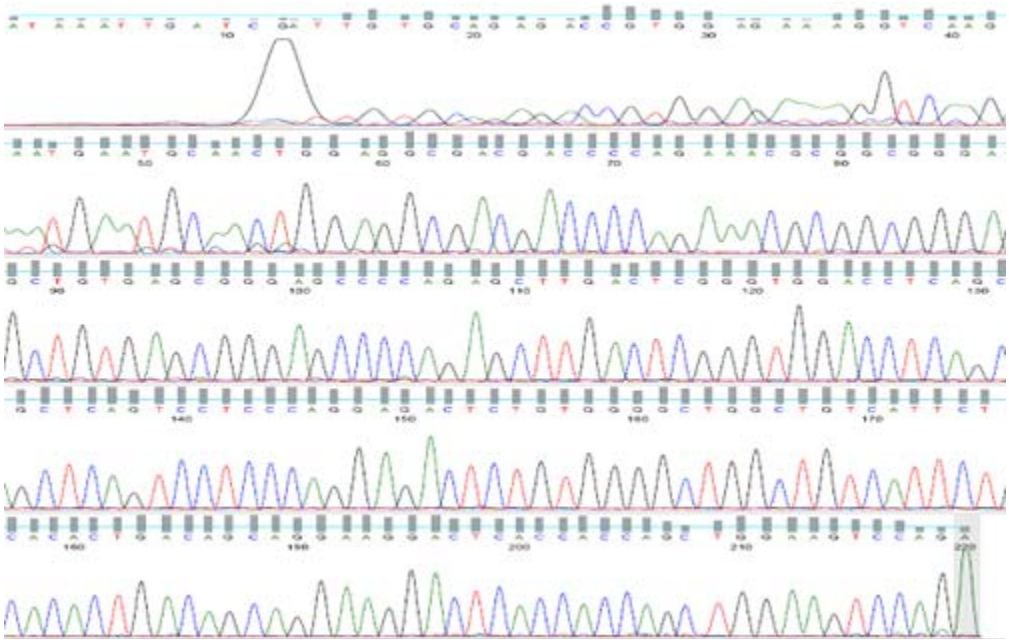

Fig. 2. Chromatogram in the parkin gene sequence

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 Score =  367 bits (406),  Expect = 3e-99
 Identities = 207/208 (99%), Gaps = 1/208 (0%)
 Strand=Plus/Plus

Query  13   ATTGTGCAGAGACCGTGGAGAAA-GGTCAAGAAATGAATGCAACTGGAGGCGACGACCCC  71
            ||||||||||||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  306  ATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATGAATGCAACTGGAGGCGACGACCCC  365

Query  72   AGAAACGCGGCGGGAGGCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCTCAGC  131
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366  AGAAACGCGGCGGGAGGCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCTCAGC  425

Query  132  AGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCTGTCATTCTGCACACTGACAGCAGG  191
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426  AGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCTGTCATTCTGCACACTGACAGCAGG  485

Query  192  AAGGACTCACCACCAGCTGGAAGTCCAG  219
            ||||||||||||||||||||||||||||
Sbjct  486  AAGGACTCACCACCAGCTGGAAGTCCAG  513

>ref|NM_004562.1| UEGM Homo sapiens Parkinson disease (autosomal recessive, j
2, parkin (PARK2), transcript variant 1, mRNA
Length=2960

 GENE ID: 5071 PARK2 | Parkinson disease (autosomal recessive, juvenile) 2,
parkin [Homo sapiens] (Over 100 PubMed links)

 Score =  367 bits (406),  Expect = 3e-99
 Identities = 207/208 (99%), Gaps = 1/208 (0%)
 Strand=Plus/Plus

Query  13   ATTGTGCAGAGACCGTGGAGAAA-GGTCAAGAAATGAATGCAACTGGAGGCGACGACCCC  71
            ||||||||||||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  306  ATTGTGCAGAGACCGTGGAGAAAAGGTCAAGAAATGAATGCAACTGGAGGCGACGACCCC  365

Query  72   AGAAACGCGGCGGGAGGCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCTCAGC  131
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366  AGAAACGCGGCGGGAGGCTGTGAGCGGGAGCCCCAGAGCTTGACTCGGGTGGACCTCAGC  425

Query  132  AGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCTGTCATTCTGCACACTGACAGCAGG  191
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426  AGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCTGTCATTCTGCACACTGACAGCAGG  485
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Fig. 3. Sequencing confirmed that the RT-PCR product was recognized as Parkin

induction of this disorder. It is suitable for the analysis of large patient groups, and it may become the basis for a diagnostic test.

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