Assessment of Genetic mutations in genes alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2 induced Parkinson's disease (PD) in patients Tabriz,IRAN

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Abstract

In this study we have analyzed 56 people. 26 patients Parkinson's disease (PD) and 30 persons control group.The genes alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2 analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with nervous disorders, Parkinson's disease (PD). In fact, of all people with Parkinson's disease (PD), 160 Parkinson's disease (PD) had a genetic mutation in the genes alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin) And 107 Parkinson's disease (PD) had a genetic mutations in PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2 respectively. Any genetic mutations in the target genes control group, did not show.

Key Words: Genetic study, Parkinson's disease (PD),Mutations The genes alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2.

Introducation

Today, neurological disorders, neuromuscular disorders is very important in creating. Including neurological disorders, including Parkinson's disease. Parkinson's disease is a neuromuscular disorder that commonly causes hand tremors and shaking head. Parkinson's disease is caused by genetic mutations, but also epigenetic factors are critical in inducing the disease.

Parkinson's disease (**PD**) is a long-term degenerative disorder of the central nervous system that mainly affects the motor system.[1] The symptoms generally come on slowly over time. Early in the disease, the most obvious are shaking, rigidity, slowness of movement, and difficulty with walking.[1] Thinking and behavioral problems may also occur. Dementia becomes common in the advanced stages of the disease. Depression and anxiety are also common occurring in more than a third of people with PD.[2] Other symptoms include sensory, sleep, and emotional problems.[1][2] The main motor symptoms are collectively called "parkinsonism", or a "parkinsonian syndrome".[3][4]

The cause of Parkinson's disease is generally unknown, but believed to involve both genetic and environmental factors. Those with a family member affected are more likely to get the disease themselves.[4] There is also an increased risk in people exposed to certain pesticides and among those who have had prior head injuries while there is a reduced risk in tobacco smokers and those who drink coffee or tea.[4][5] The motor symptoms of the disease result from the death of cells in the substantia nigra, a region of the midbrain. This results in not enough dopamine in these areas.[1] The reason for this cell death is poorly understood, but involves the build-up of proteins into Lewy bodies in the neurons.[4] Diagnosis of typical cases is mainly based on symptoms, with tests such as neuroimaging being used to rule out other diseases.[1]

There is no cure for Parkinson's disease.[1] Initial treatment is typically with the antiparkinson medication L-DOPA (levodopa), with dopamine agonists being used once levodopa becomes less effective. As the disease progresses and neurons continue to be lost, these medications

become less effective while at the same time they produce a complication marked by involuntary writhing movements.[2] Diet and some forms of rehabilitation have shown some effectiveness at improving symptoms.[6][7] Surgery to place microelectrodes for deep brain stimulation has been used to reduce motor symptoms in severe cases where drugs are ineffective.[1] Evidence for treatments for the non-movement-related symptoms of PD, such as sleep disturbances and emotional problems, is less strong.[4]

In 2013, PD was present in 53 million people and resulted in about 103,000 deaths globally.[8][9] Parkinson's disease typically occurs in people over the age of 60, of which about one percent are affected.[1][10] Males are more often affected than females.[4] When it is seen in people before the age of 40 or 50, it is called young onset PD.[11] The average life expectancy following diagnosis is between 7 and 14 years.[2] The disease is named after the English doctor James Parkinson, who published the first detailed description in *An Essay on the Shaking Palsy,* in 1817.[12][13] Public awareness campaigns include World Parkinson's Day (on the birthday of James Parkinson, 11 April) and the use of a red tulip as the symbol of the disease.[14] People with parkinsonism who have increased the public's awareness of the condition include actor Michael J. Fox, Olympic cyclist Davis Phinney, and late professional boxer Muhammad Ali.[15][16][17]

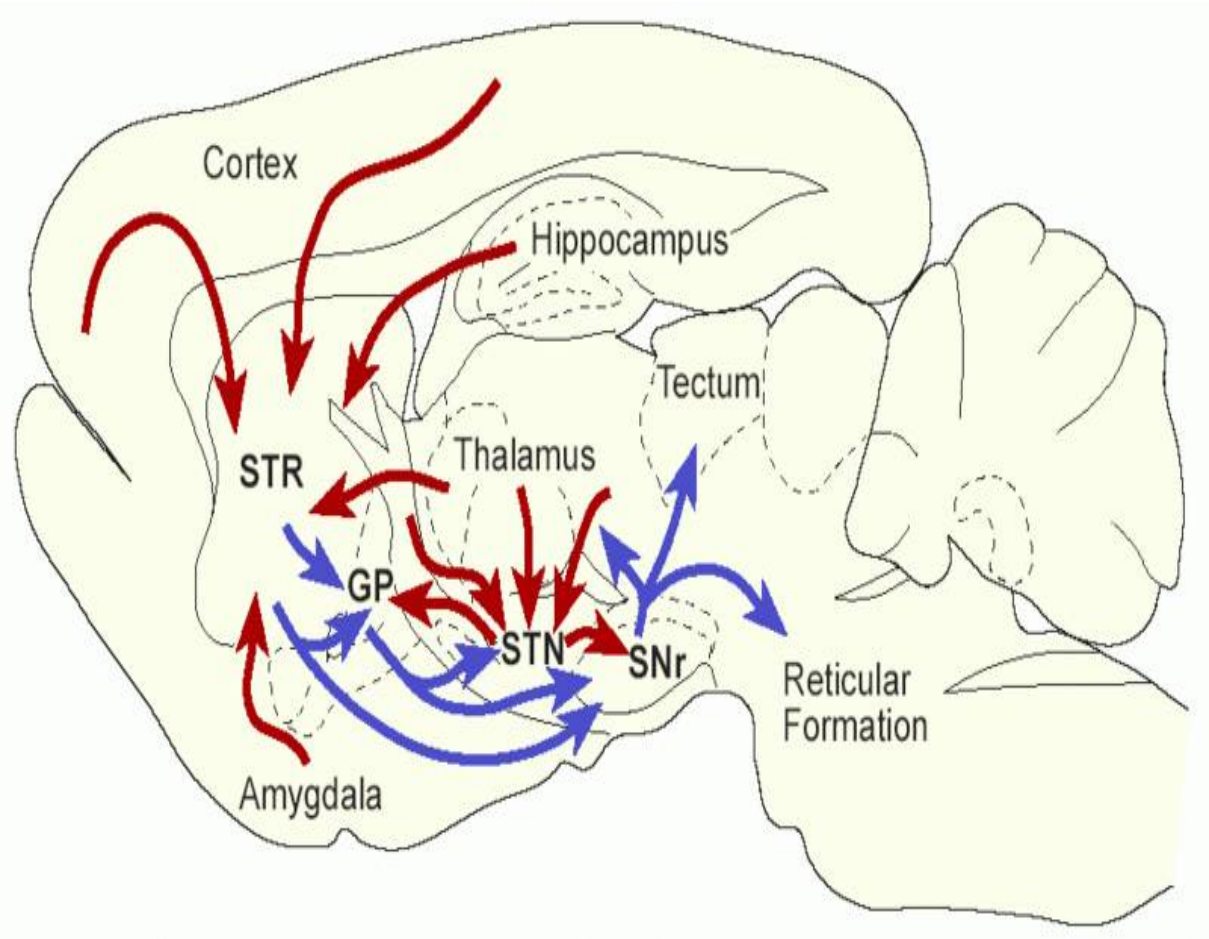


Figure 1: Schematic view of the structure of the cerebral cortex and hippocampus of the human brain.

Materials and Methods

In this study,26 patients with Parkinson's disease (PD) and 30 control group were studied. Peripheral blood samples from patients and parents with written permission control was prepared.After separation of serum, using Real Time-PCR technique of tRNA molecules were collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solutionin ratio of 1to5with the peripheral blood of patients and controls were mixed.After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 minat 400 Gera. The cells ediment with PBS (phosphate buffered saline), pipetajh and slowly soluble carbohydrate ratio of 1to2onficole(Ficol)was pouredin the 480G was centrifuged for 34 minutes.Mono nuclear Neuroglial cells also are included,has alower density than ficole and soon which they are based.The remaining erythrocytes has a molecular weight greater than fico leand deposited in test tubes.

The supernatant, which contained the mono nuclear cells was removed, and the 400 Gera was centrifuged for 12 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 34 minutes incubation at 5 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription genes alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2
,and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose,approximately 4-5 × 10^3 Neuroglial cells were transfer red to1.5ml Eppendorf tube and then was centrifuged at 2000 rpm for 7minutes at time. Remove the supernatant culture medium and there maining sediment, 100µl of PBS buffer was added. After adding 5-10µl $CD4^+$ PE monoclonal anti body to the cell suspension for 60 min at 4°C, incubated and read immediately by flow cytometry. For example,rather than control anti body Neuroglial cells PE,IgG1 negative control solution was used.

Total mRNA extraction proced urein cludes:
1) 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200µl chloroform solution to target mix, then transfer the micro tubes were added,and the shaker well was mixed for 15 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4°C an was centrifuged at 13200 rpm era. Remove the upper phase product were transfer reductase new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C were incubated.

2)Then for 45 min at 4°Can was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C an by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placedat room

temperature until completely dry deposition. The precipitate was dissolved in 20µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

To assessment the quality of mi-RNAs,the RT-PCR technique was used. The cDNA synthesis inreverse transcription reaction(RT)kit(Fermentas K1622) and 1µloligoprimers18(dT) was performed. Following the PCR reaction2µM dNTP, 1µg cDNA,FermentasPCR buffer1X, 0 / 75µM MgCl2, 1.25 U / µL Tag DNA at 95°C for 4 min, 95°C for 30s, annealing temperature58°C for 30s, and72 °C for30 seconds, 35 cycles were performed. Then1.5% agarose gel,the PCR product was dumped in wells after electrophores is with ethidium bromide staining and color were evaluated.

Findings

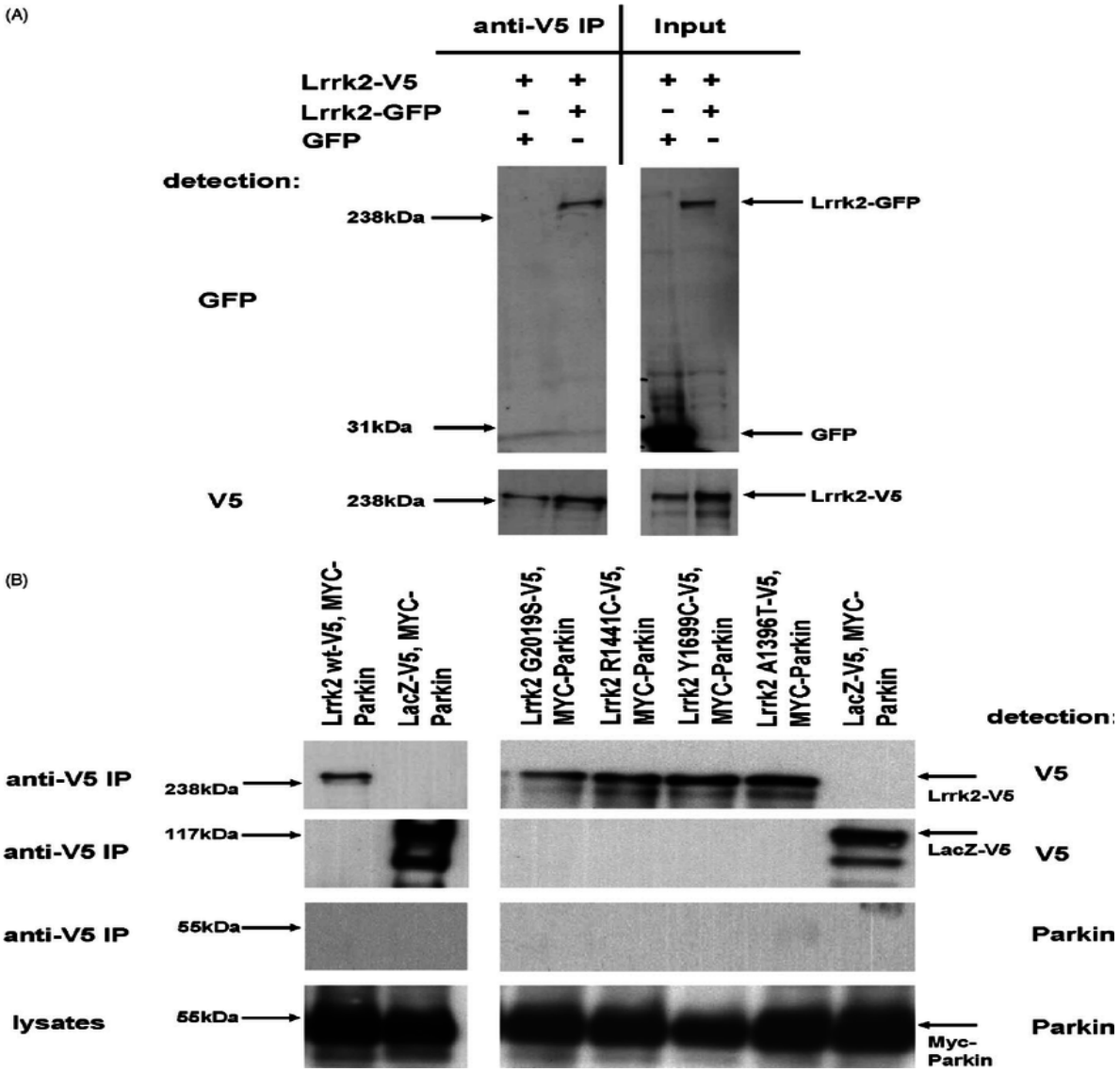


Figure 2: Schematic view of the pattern formed in the band PARKIN and LRRK2gene in patients with Parkinson's.

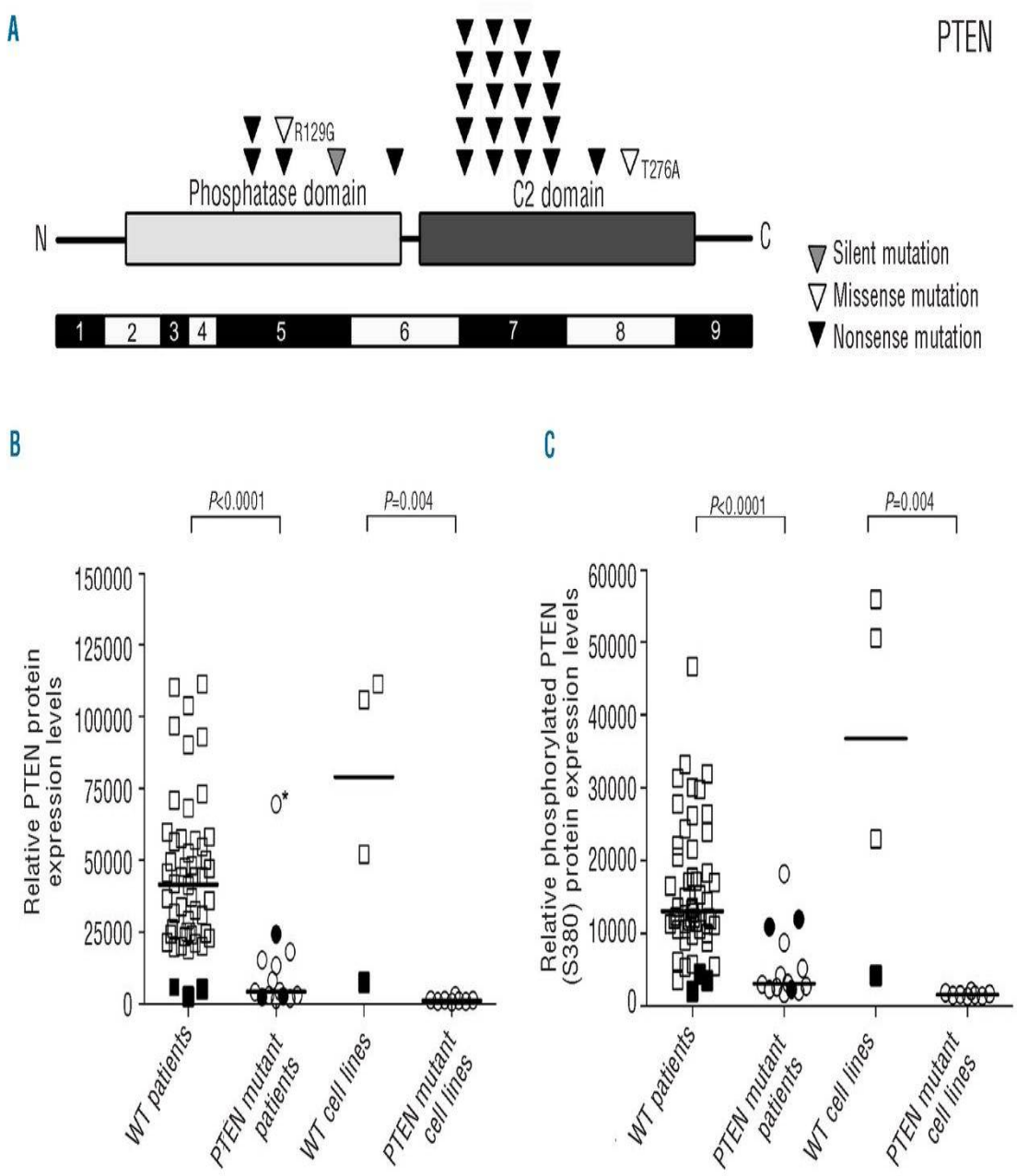


Figure 3: Schematic view of the PTEN gene expression in cells with mutated in patients with Parkinson's disease.

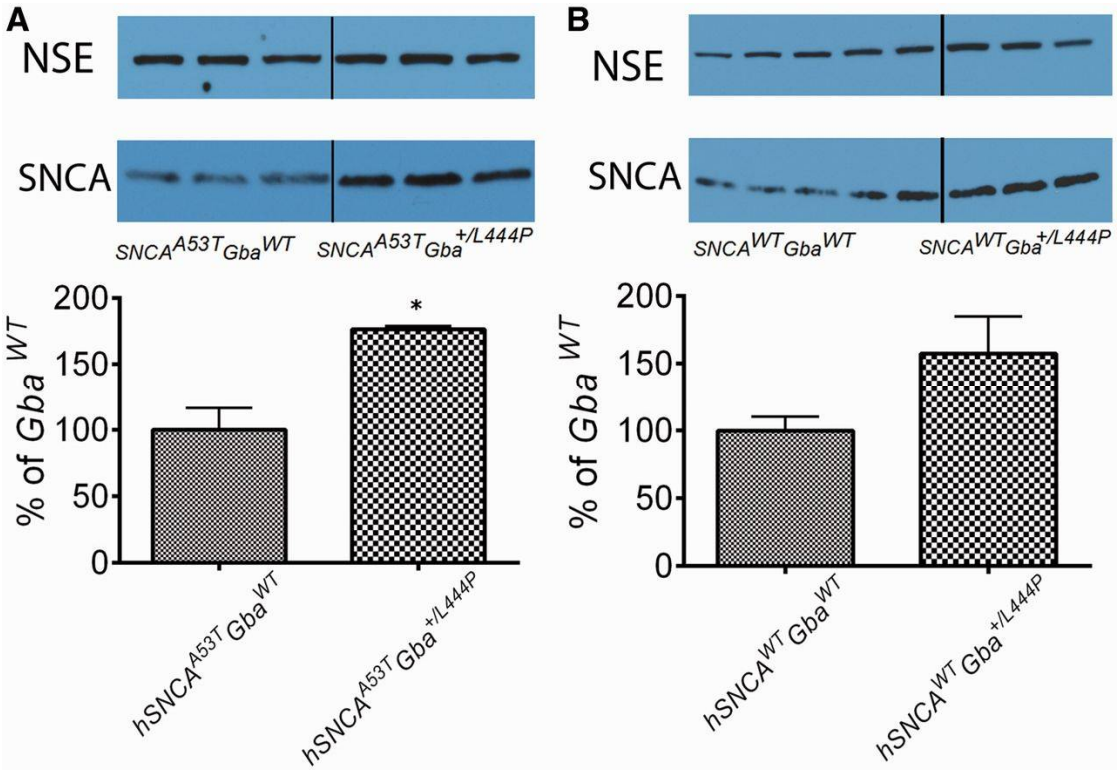


Figure 4: Schematic view of pattern formation of the band in the gene SNCA along with a diagram of cells mutated in patients with Parkinson's disease.

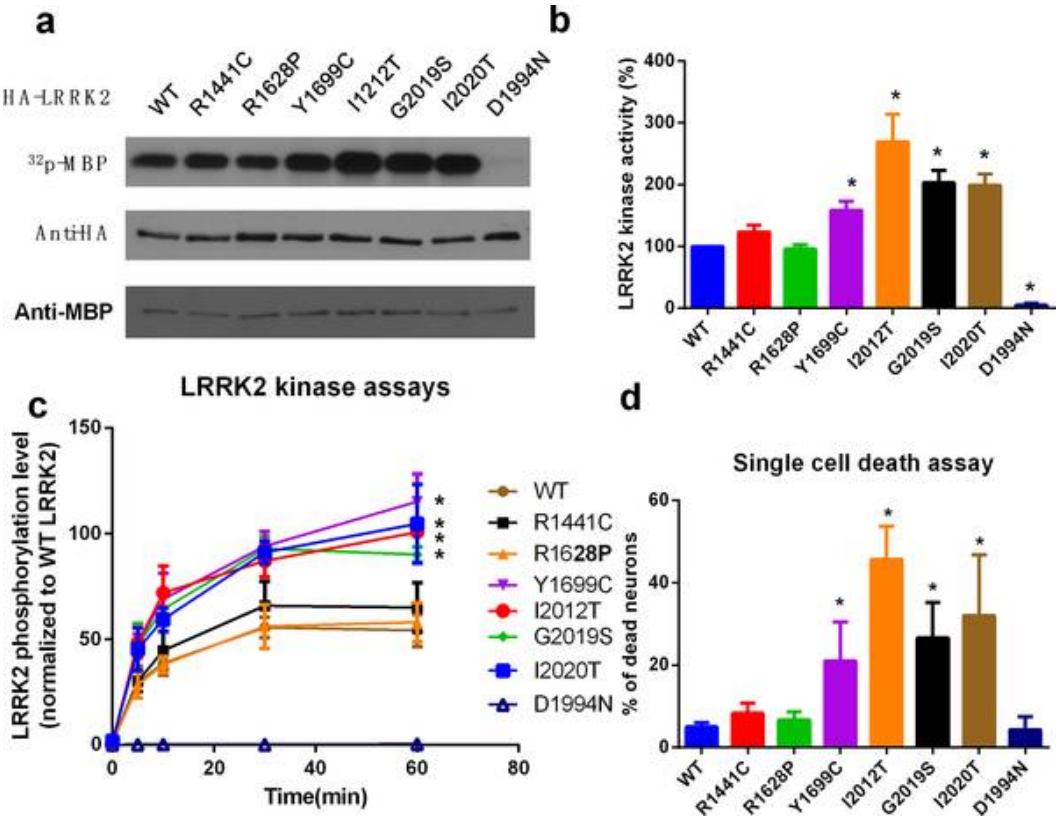


Figure 5: schematic diagram of epigenetic pattern with phosphorylation of LRRK2 gene gang of mutant cells in patients with Parkinson's disease.

Discussion and conclusion

According to the results of sequencing the genome of patients with Parkinson's disease (PD), and the genetic mutations alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2.genes found that about 94% of patients with Parkinson's disease (PD), they have this genetic mutations. Patients with Parkinson's disease (PD), unusual and frightening images in the process of Parkinson's disease (PD), experience. Lot epigenetic factors involved in Parkinson's disease (PD). But the most prominent factor to induce Parkinson's disease (PD), mutations is alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2 genes.This genes can induce the birth and can also be induced in the adulthood.

Acknowledgments

Thanks to everyone who helped us in doing this project, very grateful. Patients and their families also accept patients who had very much for your cooperation in this study.

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