

Research Article

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

Shahin Asadi^{1*}, Mahsa Jamali¹, Samaneh Sadeh Dell²

¹Molecular Genetics, Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran

²Molecular Biology Genetics, Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran

***Corresponding author:** Shahin Asadi, Molecular Genetics, Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran, Tel: +98 9379923364; Email: shahin.asadi1985@gmail.com

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Abstract

In this study, we have analyzed total 56 persons. Where 26 people are with Parkinson's Disease (PD) and 30 people are control group. The genes alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase2 (LRRK2 or dardarin), PTEN-Induced Putative Kinase1 (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 Kinase2 (LRRK2 or dardarin) And 107 Parkinson's Disease (PD) had a genetic mutation in PTEN-Induced Putative Kinase1 (PINK1), DJ-1 and ATP13A2 respectively. Any genetic mutations in the target genes control group, did not show.

Keywords: Genetic study; leucine-rich repeat kinase 2 (LRRK2 or dardarin); Mutations The genes alpha-synuclein (SNCA); Parkinson's Disease (PD); parkin (PRKN); PTEN-Induced Putative Kinase 1 (PINK1); DJ-1 and ATP13A2.

Introduction

Today, neurological disorders, neuromuscular disorders are 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].

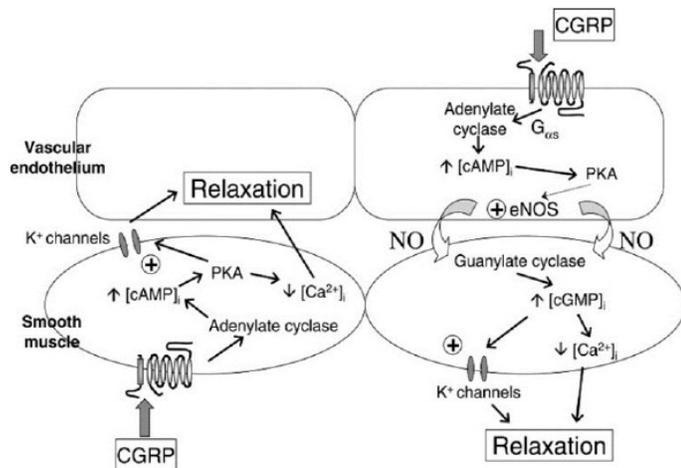


Figure 1: Schematic view of the structure of the cerebral cortex and hippocampus of the human brain.

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 solution in ratio of 1 to 5 with 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 min at 400 Gera. The cells sediment with PBS (Phosphate Buffered Saline), pipetajh and slowly soluble carbohy-

drate ratio of 1 to 2 onficole (Ficol) was poured in 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 ficole and 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 minute's 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 kinase1 (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 × 10³ Neuroglial cells were transfer red to 1.5ml Eppendorf tube and then was centrifuged at 2000 rpm for 7 minutes at time. Remove the supernatant culture medium and the remaining 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 procedure includes:

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 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°C 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 by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at 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 in reverse transcription re-

action (RT) kit (Fermentas K1622) and 1µl oligoprimers18 (dT) was performed. Following the PCR reaction 2µM dNTP, 1µg cDNA, Fermentas PCR buffer1X, 0.75µM Mg Cl₂, 1.25 U / µL Tag DNA at 95°C for 4 min, 95°C for 30s, annealing temperature 58°C for 30s, and 72°C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophoresis with ethidium bromide staining and color were evaluated.

Findings

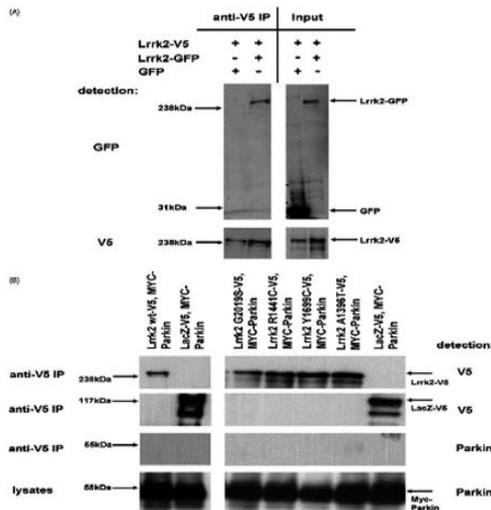


Figure 2: Schematic view of the pattern formed in the band PARKIN and LRRK2 gene in patients with Parkinson's.

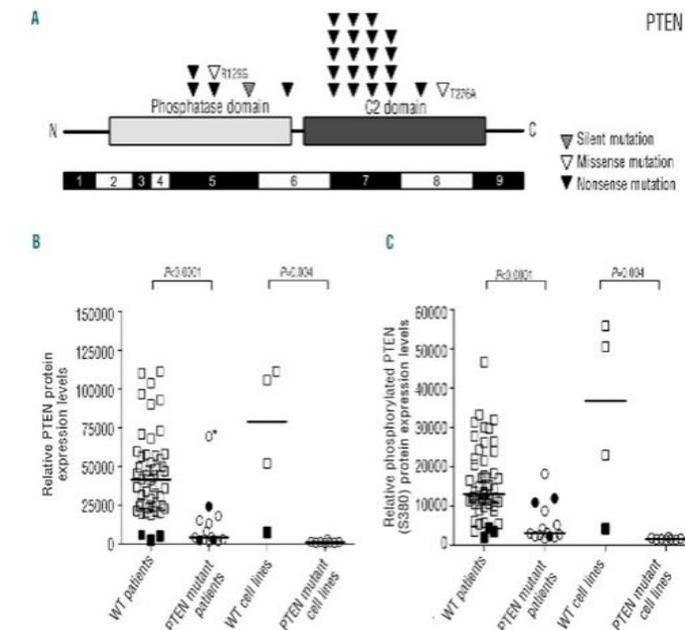


Figure 3: Schematic view of the PTEN gene expression in cells with mutated in patients with Parkinson's.

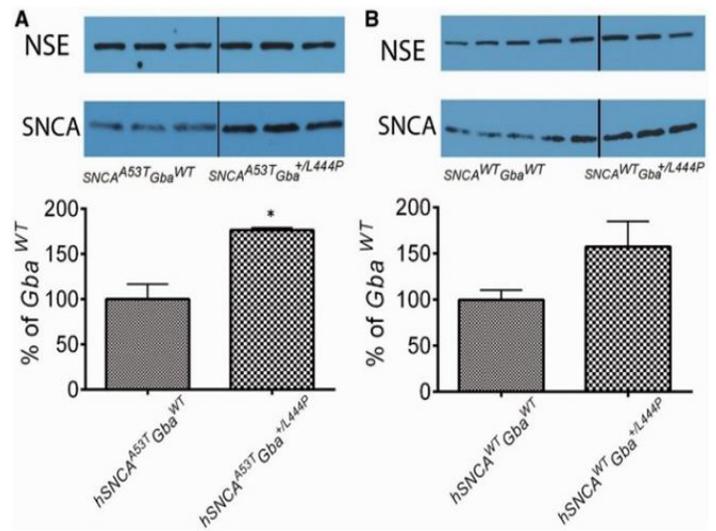


Figure 4: Schematic view of pattern formation of the band in the gene SNCA along with a diagram of cells mutated in patients with Parkinson's disease.

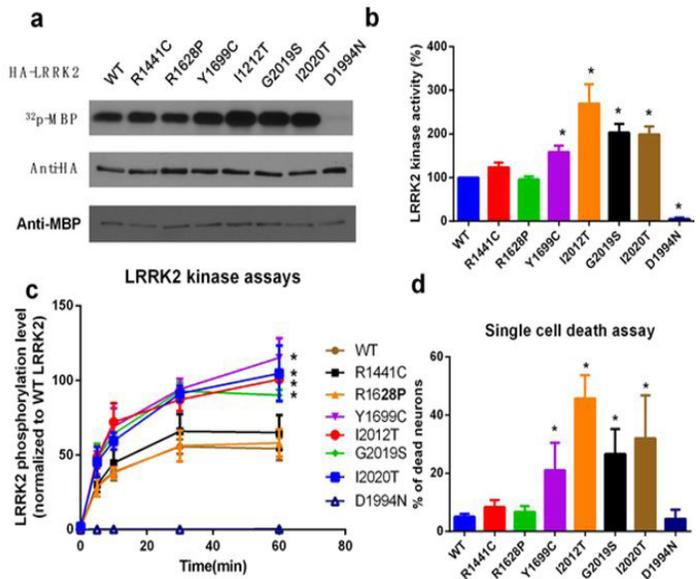


Figure 5: schematic diagram of epigenetic pattern with phosphorylation of LRRK2 gene gang of mutant cells in patients with Parkinson's disease.

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 mutation. Patients with Parkinson's Disease (PD), unusual and frightening images in

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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 kinase1 (PINK1), DJ-1 and ATP13A2 genes. These genes can induce the birth and can also be induced in the adulthood.

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References

1. Sveinbjornsdottir S (2016) The clinical symptoms of Parkinson's disease. *Journal of Neurochemistry* 1: 318-324.
2. Kalia LV, Lang AE (2015) Parkinson's disease. *Lancet* 386: 896-912.
3. Quintana B JL, Allam MF, Del Castillo AS, Navajas RF (2009) Parkinson's disease and tea: a quantitative review. *Journal of the American College of Nutrition* 28: 1-6.
4. Global Burden of Disease Study 2013, Collaborators (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013 a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386: 743-800.
5. Shulman JM, De Jager PL, Feany MB (2011) Parkinson's disease: genetics and pathogenesis. *Annual Review of Pathology* 6: 193-222.
6. Schrag A (2007) Epidemiology of movement disorders. Parkinson's disease and movement disorders. Hagerstown Maryland: Lippincott Williams & Wilkins. 50-66.
7. Barnett-Cowan M, Dyde RT, Foxe SH, Moro E, Hutchison WD, et al. (2010) Multisensory determinants of orientation perception in Parkinson's disease. *Neuroscience* 167: 1138-1150.
8. Galpern WR, Lang AE (March 2006) Interface between tauopathies and synucleinopathies: a tale of two proteins. *Annals of Neurology*. 59: 449-458.
9. Aarsland D, Londos E, Ballard C (2009) Parkinson's disease dementia and dementia with Lewy bodies: different aspects of one entity. *Int. Psychogeriatr* 21: 216-219.
10. Charcot, Jean-Martin, Sigerson G (1879) Lectures on the diseases of the nervous system (Second ed.). Philadelphia: Henry C. Lea. p. 113. The strokes forming the letters are very irregular and sinuous, whilst the irregularities and sinuosities are of a very limited width. (...) the down-strokes are all, with the exception of the first letter, made with comparative firmness and are, in fact, nearly normal - the finer up-strokes, on the contrary, are all tremulous in appearance (...).
11. Cooper G, Eichhorn G, Rodnitzky RL (2008). Parkinson's disease. In Conn PM. *Neuroscience in medicine*. Totowa 508-512.
12. Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, et al. (2009) Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. *Lancet Neurol* 8: 1128-1139.
13. Fung VS, Thompson PD (2007) Rigidity and spasticity. In Tolosa E, Jankovic. *Parkinson's disease and movement disorders*. Hagerstown, Lippincott Williams & Wilkins 504-513.
14. Yao S.C, Hart A.D, Terzella M.J (2013) An evidence-based osteopathic approach to Parkinson disease. *Osteopathic Family Physician* 5: 96-101.
15. Murray ED, Buttner EA, Price BH (2012) Depression and Psychosis in Neurological Practice. In Bradley WG, Daroff RB, Fenichel GM, Jankovic J. *Bradley's Neurology in Clinical Practice: Expert Consult – Online and Print, 6e* (Bradley, Neurology in Clinical Practice e-dition 2v Set). 1 (6th ed.). Philadelphia, PA: Elsevier/Saunders. 102-103.
16. Hughes JR (2009) Absence seizures: a review of recent reports with new concepts. *Epilepsy & behavior: E&B* 15: 404-412.
17. Bradley, Walter G. (2012) *Bradley's neurology in clinical practice*. Philadelphia PA: Elsevier/Saunders.
18. Simon, David A.G, Michael J.A, Roger P (2012) *12 Clinical neurology* (8th ed.) New York: McGraw-Hill Medical.