

## Assessment of Mutation Genetics in HTT (Hi-CAG), Gene for Induced Huntington's disease in Tabriz, Iran

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### Abstract

In this study we have analyzed 120 people. 59 Huntington's disease (HD) and 61 control group. The gene HTT analyzed in terms of genetic mutation made. In this study, people who have genetic mutation were targeted, with nervous disorders, Huntington's disease (HD). In fact, of all people with Huntington's disease (HD), 59 Huntington's disease (HD) had a genetic mutation in the gene HTT Huntington's disease (HD). Any genetic mutations in the target genes control group, did not show.

**Keywords:** Genetic study; Huntington's disease (HD); Mutation The gene HTT

**Received Date:** December 01, 2016

**Accepted Date:** December 31, 2016

**Published Date:** January 06, 2017

**Citation:** Asadi, S., et al. Assessment of Mutation Genetics in HTT (Hi-CAG), Gene for Induced Huntington's disease in Tabriz, Iran. (2017) Cell Immunol Serum Biol 3(2): 93- 98.

### Introduction

Today, neurological disorders, neuromuscular disorders are very important in creating. Including neurological disorders, including Huntington's disease (HD). Huntington's disease (HD) is a neuromuscular disorder that commonly causes A progressive neurodegenerative disease with autosomal dominant inheritance, with the incidence in adulthood, the disease has three abnormal movements, cognitive disorders, psychological disorders known.. Huntington's disease (HD) is caused by genetic mutations, but also epigenetic factors are critical in inducing the disease.

Huntington's disease (huntington) is a genetic disease caused by defects in the gene and chromosome 4 caused. Huntington's is an inherited disease that damages the nerve cells in the brain. Most people who have Huntington's disease, symptoms of the disease at the age of 40 and 50 years, respectively. But sometimes sooner or later the age of onset of symptoms. If symptoms occur earlier than 20 years, it is called juvenile Huntington<sup>[1]</sup>. If we Huntington's disease based on age classification, will be divided into two categories:

**The first:** The most common symptoms of Huntington's disease after the age of 40 and 50 years old<sup>[2]</sup>.

**The second category:** a few are infected in their teens and early onset of symptoms.

If one parent has Huntington's disease, Huntington's likely that their children will be affected, 50 percent<sup>[3]</sup>.

If a person does not receive the defective gene from their parents, it could pass the gene to their children. But if a child who has one parent has Huntington, Huntington's gene, they are not; it does not transmit to their children<sup>[4]</sup>.

### Symptoms of Huntington's disease

**Movement Disorders:** It can be both voluntary movements and involuntary movements will be included. Impairment of voluntary movements may have a greater impact on the ability of the individual and his relations with others interfere<sup>[5,6]</sup>. Movement disorders, including the following cases:

Jerking

Contraction involuntary and sustained muscle (dystonia)

Muscle rigidity

Slow and uncoordinated movements

Abnormal eye movements slow

Difficulty walking and balance

Speech disorder

Swallowing Food

Cognitive Disorders:

Inability to start or conversation

Disruption to prioritize tasks, and organizing sub program

Lack of flexibility

Lack of impulse control, which could lead to flooding desires.

Act without thinking and moral issues and sexual problems<sup>[7,8]</sup>.  
 Impaired perception of space that can be used to take the lead so that if a person is clumsy.  
 Difficulty concentrating on one task for a long time  
 Slowness in processing thoughts and find the words  
 Difficulty and impairment in learning new information.

**Psychiatric Disorders:** The most important and most common mental disorders in Huntington's disease, depression. Symptoms of depression include:

Feel grief  
 Loss of interest in normal daily activities and  
 Insomnia or sleeping too much  
 Severe fatigue and loss of energy  
 Feelings of worthlessness and guilt  
 Disruption of the decision, distractibility and decreased concentration  
 Thoughts that may lead to suicide  
 Changes in appetite  
 Reduction or change in sexual desire, sexual behavior inappropriate.

#### Other mental disorders:

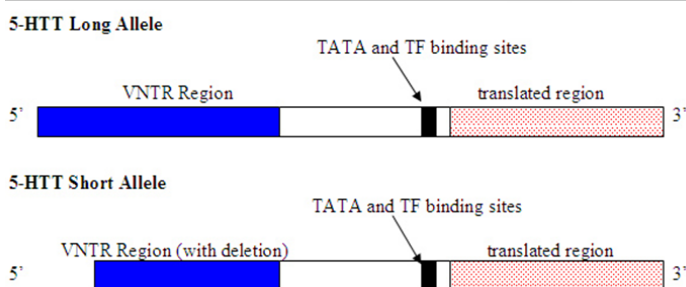
Obsessive-compulsive disorder: intrusive thoughts and repetitive behaviors  
 Mania or manic mood that is bad temper  
 Lack of motivation and lack of interest  
 Anxiety

#### Symptoms of Huntington's disease in adolescence

Initiation and progression of Huntington's disease in adolescents and young people is a little different than adults. Problems such as:  
 The loss of what has already been learned and the skills that person has earned  
 Quick academic failure  
 behavioral problems  
 Stiff and rigid muscles in children with Huntington's walk is evident  
 Changes in motor skills such as handwriting  
 The latest tremor and involuntary movements  
 Seizure

#### Treatment of Huntington's disease

There is no cure for this disease. The sole purpose of medical treatment, slowing the disease and its symptoms<sup>[9-14]</sup>.  
 Prevention of Huntington's disease  
 Genetic counseling is the best way to prevent the disease.



**Figure 1:** Schematic view of the HTT gene with long and short alleles.

## Materials and Methods

In this study, 59 patients with Huntington's disease (HD), and 60 control groups were studied. Peripheral blood samples from patients and parents with written permission control were 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 17 min at 450 Gera. The cell sediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1 to 2 on ficole (Ficol) was poured in the 780G was centrifuged for 24 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes have 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 19 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 gene HTT, and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 4 - 5 × 10<sup>3</sup> Neuroglial cells were transferred to 1.5 ml Eppendorf tube and then were centrifuged at 2000 rpm for 7 minutes at time. Remove the supernatant culture medium and there mining sediment, 100 µl of PBS buffer was added. After adding 5 - 10 µl 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.

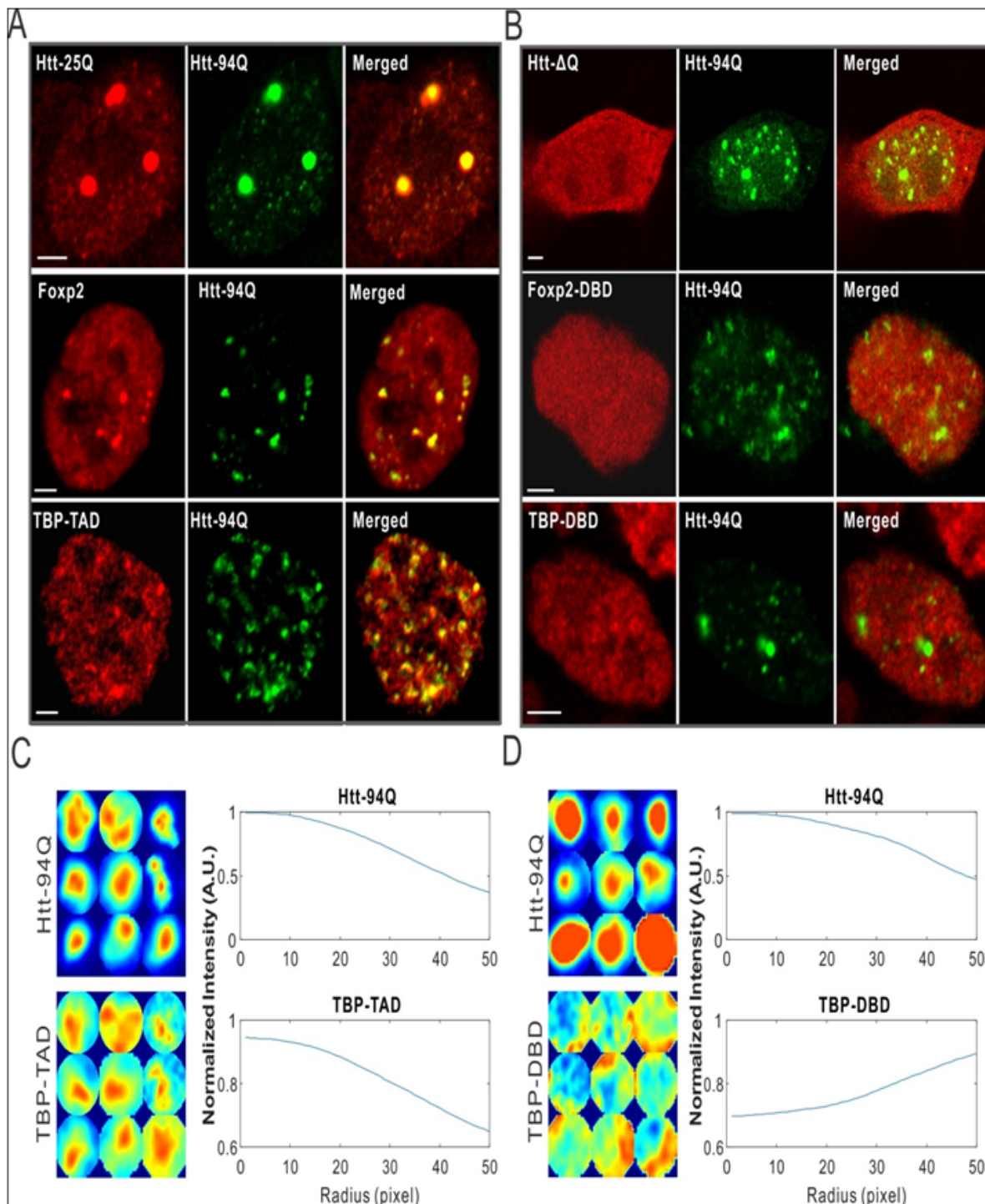
#### Total mRNA extraction procedure includes:

1 ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200 µl chloroform solutions to target mix, and 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 and was centrifuged at 13200 rpm era. Remove the upper phase product was transferred to a new microtube and to the one times the volume of cold ethanol was added. The resulting mixtures for 24 hours at -20°C were incubated.

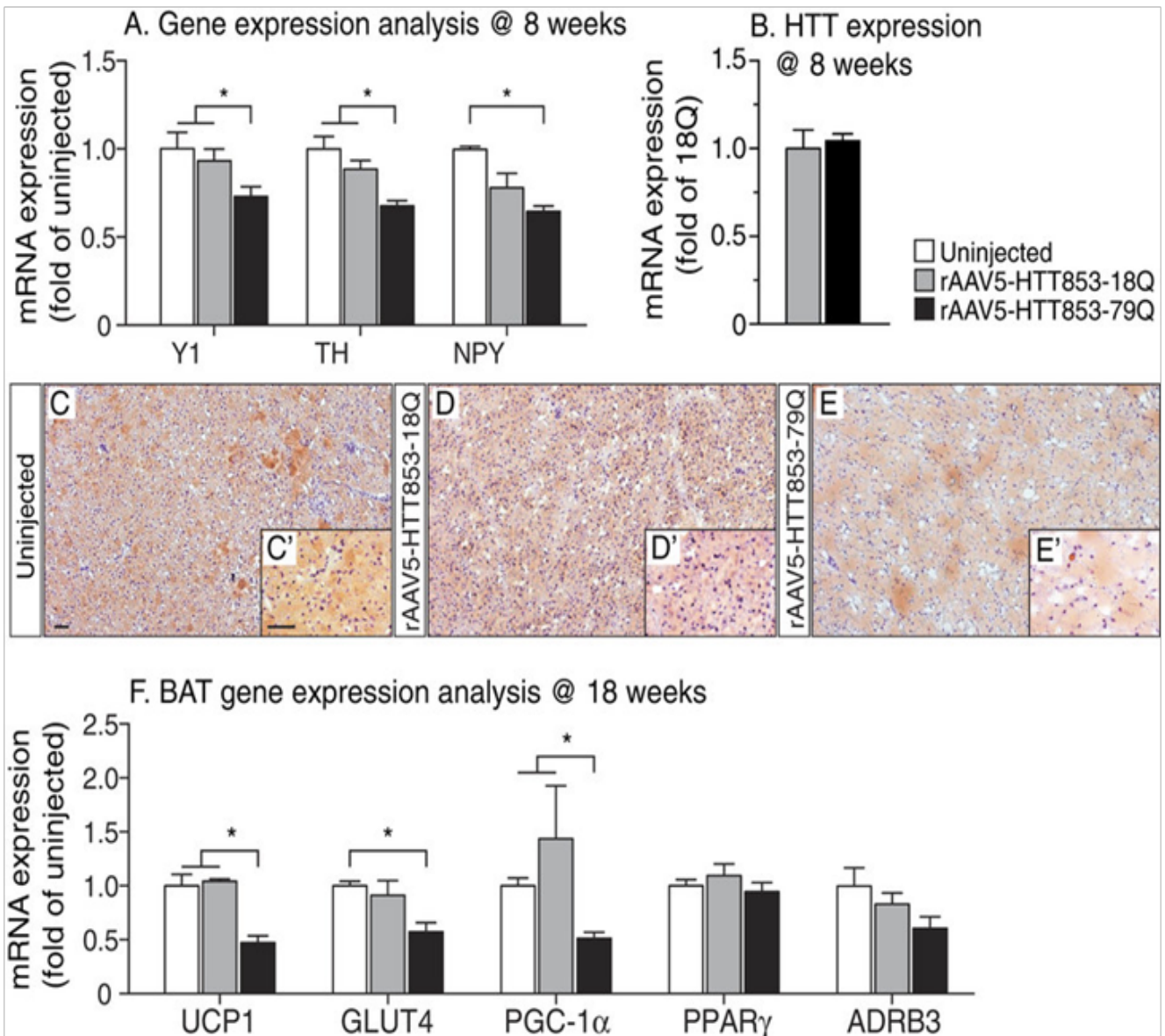
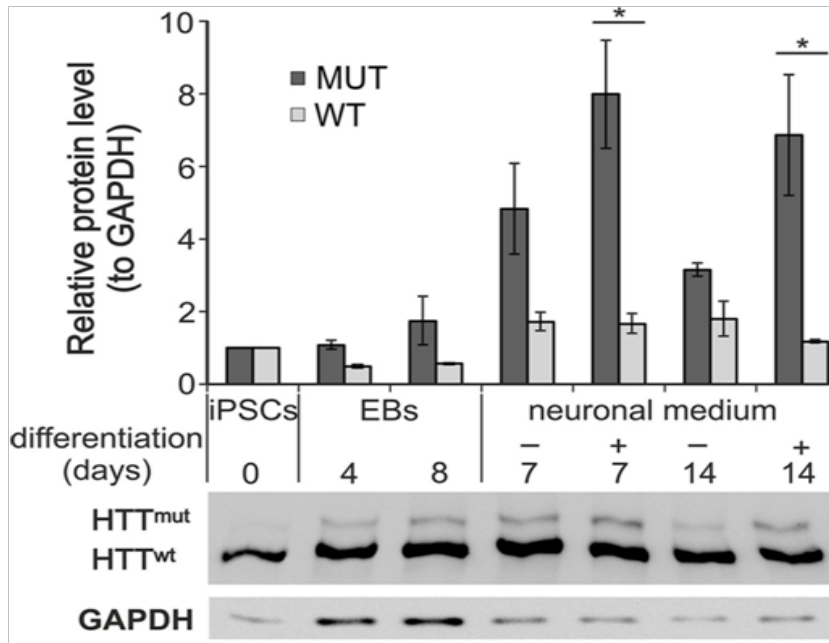
Then for 45 min at 4°C and was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C and by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20 µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

To assessment the quality of mi-RNAs, the Real Time-PCR technique was used. The cDNA synthesis in reverse tran-

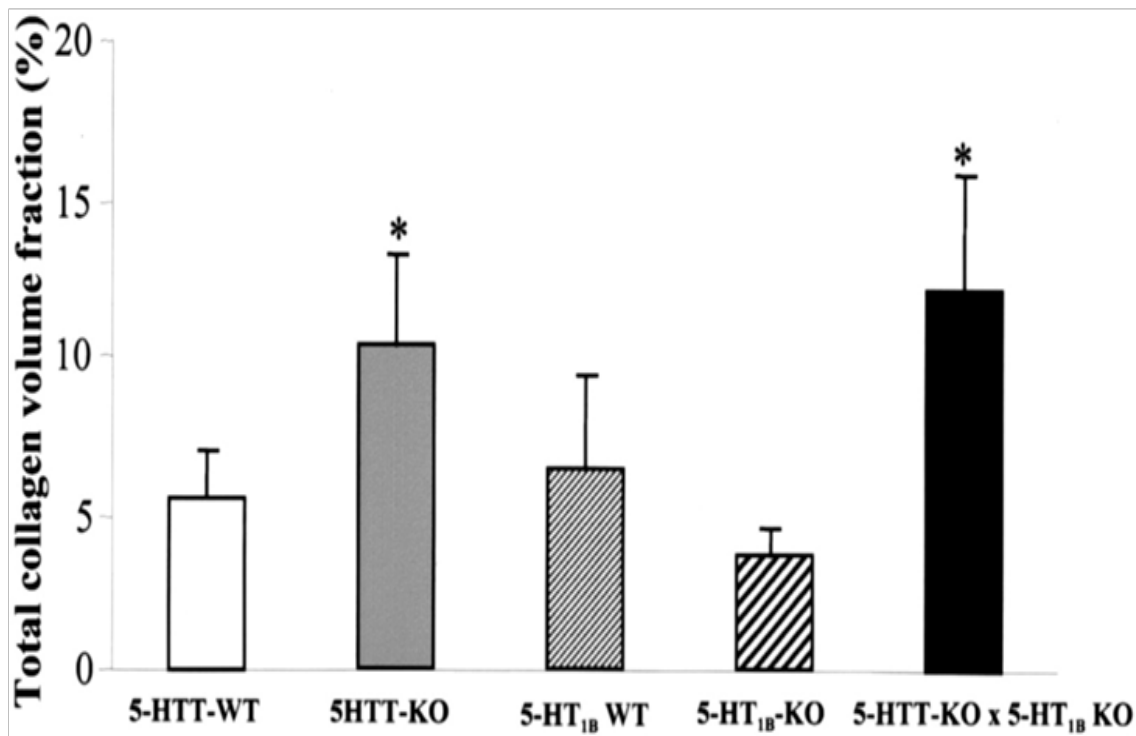
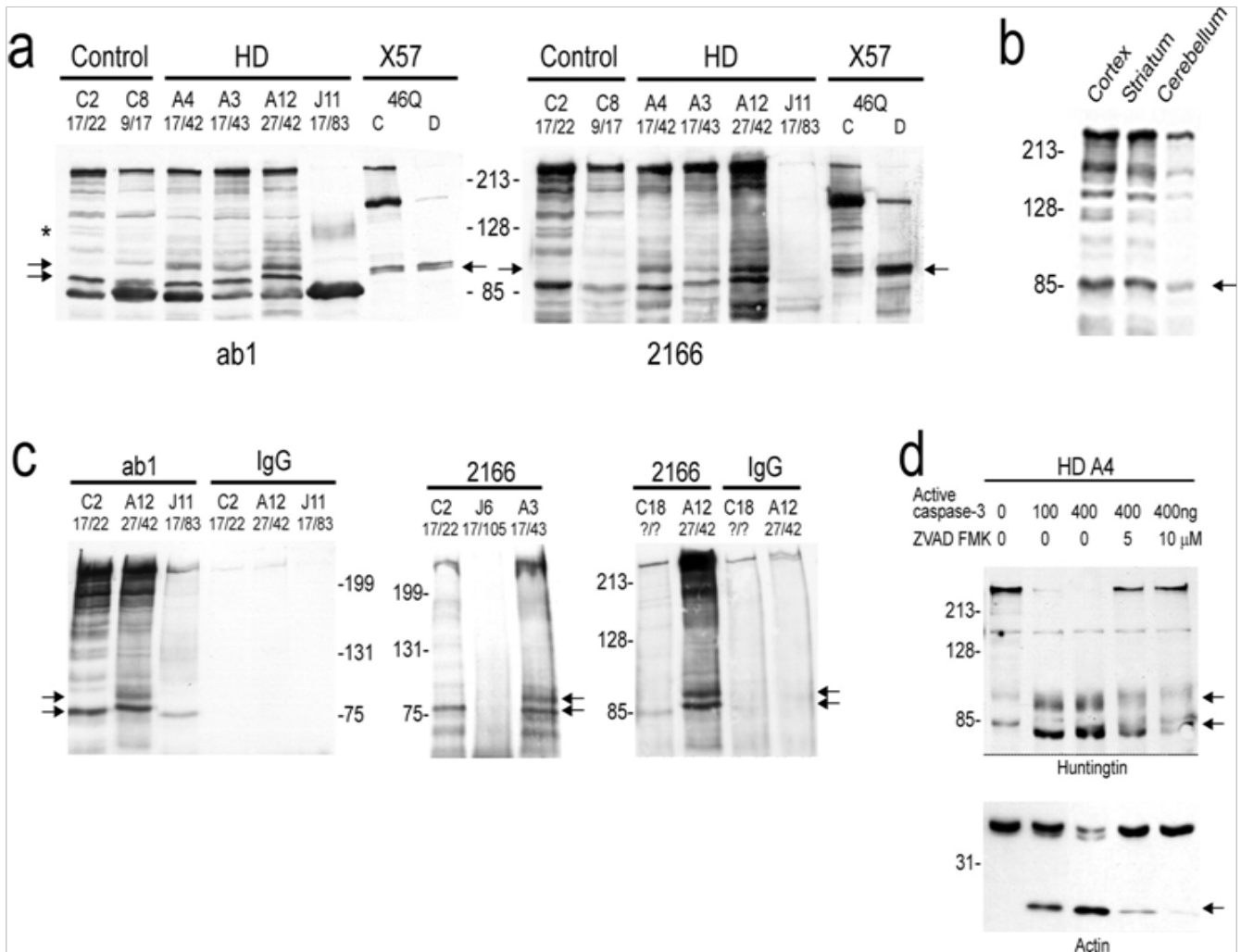
scription reaction (RT) kit (Fermentas K1622) and 1  $\mu$ l oligoprimers18 (dT) was performed. Following the PCR reaction 2  $\mu$ M dNTP, 1  $\mu$ g cDNA, Fermentas PCR buffer 1X, 0 / 75  $\mu$ M MgCl<sub>2</sub>, 1.25 U /  $\mu$ L Tag DNA at 95°C for 4 min, 95°C for 30s, annealing temperature 58°C 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 was evaluated.



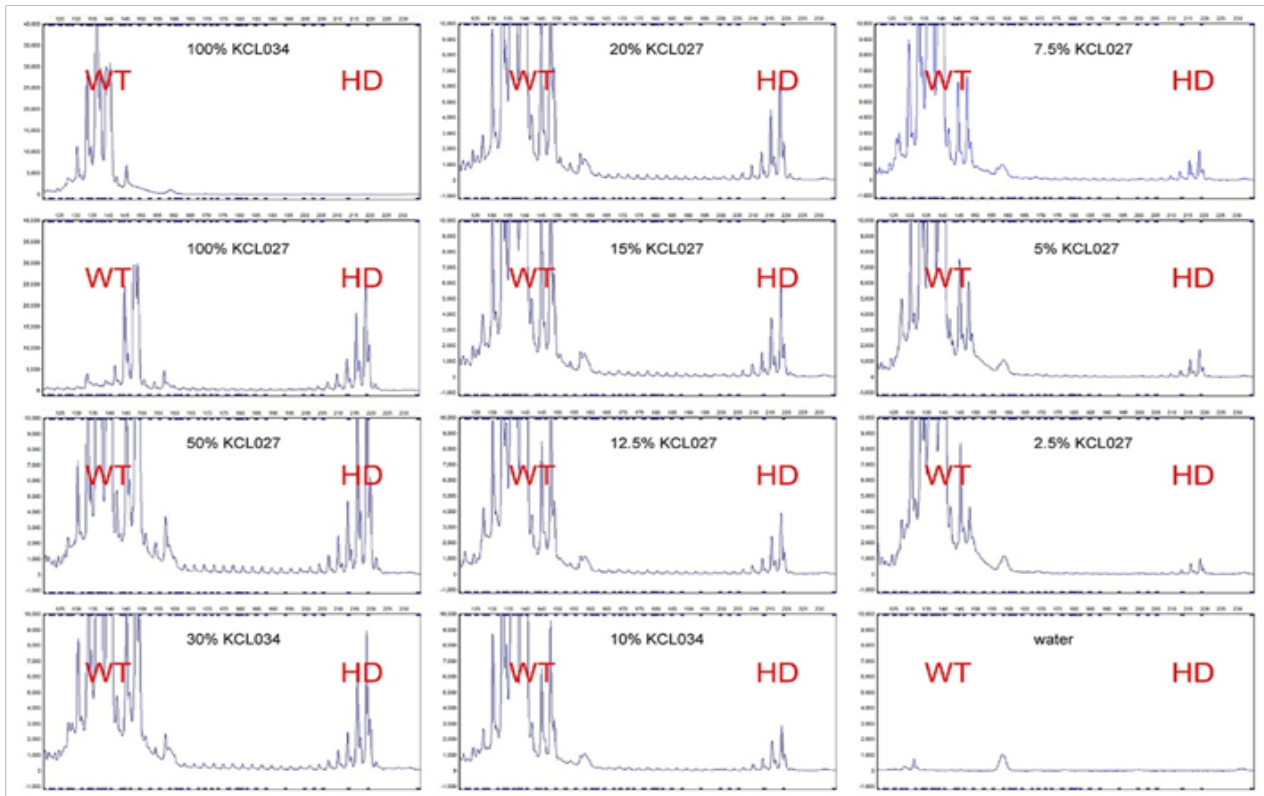
**Figure 2:** Microscopic view of HTT gene and brain imaging in patients with Huntington's statistical charts.



**Figure 3:** Schematic view of the pattern formed in the band HTT gene with genetic mutations with chart diagram of the in patients Huntington's and control group.



**Figure 4:** Schematic view of the pattern formed HTT gene and immunoglobulin gamma-band frequency diagram charts with mutations in the HTT gene in Huntington's patients and the control group.



**Figure 5:** Schematic view of the EEG in patients with Huntington's disease with HTT gene mutation compared with the control group.

## Discussion and Conclusion

According to the results of sequencing the genome of patients with Huntington's disease (HD), and the genetic mutation HTT gene found that about 99% of patients with Huntington's disease (HD), they have these genetic mutations. Patients with Huntington's disease (HD), unusual and frightening images in the process of Huntington's disease (HD), experience. Lot epigenetic factors involved in Huntington's disease (HD). But the most prominent factor to induce Huntington's disease (HD), mutation is HTT gene. This gene can induce the birth and can also be induced in the adulthood.

**Acknowledgment:** 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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