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Molecular detection of Huntington disease in patients using the PCR-based *HD* gene detection

Mana Zakeri[1], Akram Vahidi[2],Seyede Mahsa Mousavikia[3], Mohamad Taghi Akbari[4*]

[1]MSc, Department of Biology, Tehran Medical Branch, Islamic Azad University, Tehran, Iran.

[2]Golestan University of Medical Sciences, Gorgan, Iran.

[3]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat modares University, Tehran, Iran

[4]PhD, Tehran Medical Genetic Laboratory, Tehran, Iran

ORCID:

0000-0002-5936-957X

0000-0002-0399-3824

0009-0001-0345-5997

0000-0002-3305-4967

Corresponding author: Mohamad Taghi Akbari , Tehran Medical Genetics Laboratory, Tehran, Iran. **Tel:** +989121598407. **Email address:** akbarimt@gmail.com.

Abstract

Background: Huntington's disease is a condition that stops parts of the brain working properly over time. It's passed on (inherited) from a person's parents. It gets gradually worse over time and is usually fatal after a period of up to 20 years. *HD* gene may affect the pathobiology of the disease. The present study was aimed to determine the Huntington's disease amongst the patients with common clinical signs of the disease using the polymerase chain reaction. **Methods:** Peripheral blood samples were taken from 112 patients with the clinical signs of the Huntington's disease. DNA was extracted from blood samples and the presence of *HD* gene was evaluated using the PCR. **Results:** In examined patients, 67 men and 45 women were observed and a family history of Huntington's disease was seen in 25 patients. Of the 112 patients, 56 (50%) had *HD* gene in the PCR and were recognized as Huntington's disease. Transmission of paternal mutant alleles has been observed in all these patients. **Conclusion:** *HD* gene detection maybe an applied method to identify the Huntington's disease among patients with the clinical signs. However, further clinical and laboratory-based investigations should perform to assess the exact role of HD gene for identification of Huntington's disease.

Keywords: Huntington's disease, HD gene, PCR.

Introduction

Huntington disease is a progressive neurodegenerative disorder that belongs to a unique group of autosomal-dominant disorders. This disorder is caused by CAG trinucleotide repeats in the 5′ coding region of the Interesting Transcript15 (IT15) gene located on locus 4p16.3[1]. Huntington disease expanded alleles have more than 36 CAG units in the *HD* gene, whereas normal individuals have from 10–35 CAG units[2]. This mutation generates a functionally defective protein called huntingtin (HTT), a protein of uncertain molecular function(s)[3]. HTT is a ubiquitously expressed protein that is located throughout the body. Mutant HTT, which contains pathologically extended polyglutamines, causes the earliest and most dramatic neuropathological changes in the neostriatum and cerebral cortex[4], whereas a loss of wild-type HTT function contributes to disease development[5, 6].

HD gene is recognized as the best approach for detection and identification of Huntington disease among patients[7]-12.

According to the high importance of molecular and accurate diagnosis of Huntington disease, the present survey was conducted to assess the distribution of Huntington disease among patients with common clinical signs using the HD-based Polymerase Chain Reaction (PCR) in blood samples.

Materials and methods

Study procedure

This study was performed on 112 patients who referred to Tehran Medical Genetics Laboratories during the years 2004 to 2013 and were suspected of Huntington's disease. In these patients, movement disorders, mental disorders, dancing movements, and etc. were observed. Blood samples were taken from all of the and presence of the *HD*-gene was assessed by PCR. Personal information of patients were kept secret.

Inclusion and exclusion criteria

All patients with the clinical signs of the Huntington's disease, such as involuntary jerking or writhing movements (chorea), muscle problems like rigidity or muscle contracture (dystonia), slow or abnormal eye movements, impaired gait, posture and balance, difficulty with speech or swallowing, were included in the study. Additionally, patients with cognitive disorders, such as difficulty organizing, prioritizing or focusing on tasks, lack of flexibility or the tendency to get stuck on a thought, behavior or action (perseveration), lack of impulse control that can result in outbursts, acting without thinking and sexual promiscuity, lack of awareness of one's own behaviors and abilities, slowness in processing thoughts or "finding" words, and difficulty in learning new information were included in the survey. Pregnant and lactating women were excluded from the survey. Family-based history of all patients were recorded.

Samples

Sampling of patients with Huntington's symptoms was performed by specialist physicians. After obtaining the consent, 4 ml of peripheral blood was taken from the subjects and poured into tubes containing anticoagulant (EDTA).

DNA extraction and quality assessment

DNA extraction was performed using the optimized saturated salt method according to the previous survey[13]. Purity (A260/A280) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA). The truth of the DNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany)[14-18].

PCR-detection of HD gene among samples

Presence of HD gene was evaluated in all DNA samples. Table 1 shows the PCR conditions used for this purpose. Veriti 96 well Thermal Cycler (Applied Biosystems) was applied. Some of the PCR products of the HD gene were subjected to sequencing.

Statistical analysis

Statistical analysis of data was performed using the SPSS software and chi-square test. $P< 0.05$ was considered significant level[19-21].

Results

Demographic characters

A total of 112 patients with the clinical signs of the Huntington's disease were considered in this study. In these patients, 67 men and 45 women were observed and in 25 patients a family history of Huntington's disease was seen. Table 2 shows the demographic characters. A number of these patients developed Huntington's disease at an early age and developed the phenomenon of

anticipation. Transmission of paternal mutant alleles has been observed in all these patients. Among the patients in whom the phenomenon of anticipation was observed, three patients were found to have an increased amplitude of CAG recurrence. Among these patients with the phenomenon of anticipation, only three patients with transmission of the disease allele from mother to child have been observed

Table 2. Demographic characters of the studied population

Properties	Frequency
Number of HD cases	56
Age of onset	37
Male/female	25:31
Familiar history	20
CAG range	39-101

HD gene detection

Patients were first screened for *HD* gene (Figure 1).

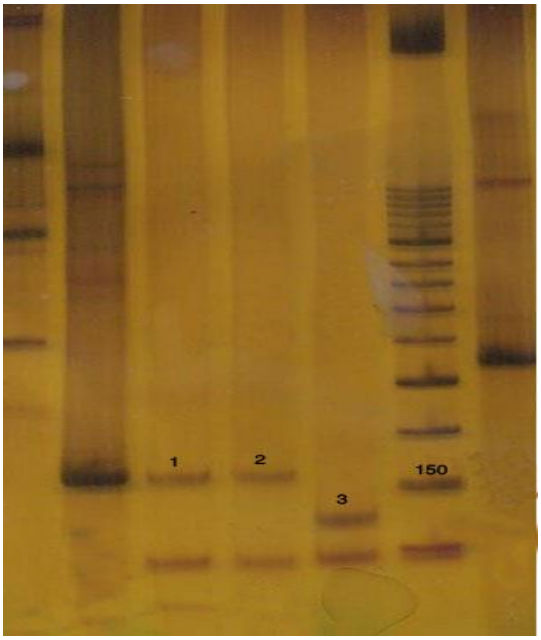

Figure 1. Findings of the gel electrophoresis of PCR products.

For this purpose, the number of CAG replicates in patients was assessed by PCR. Of the 112 patients, 56 (50%) had Huntington's disease and another 56 patients did not have an increase in the number of repeats in the IT15 gene despite the Huntington-like clinical sign. Figure 2 shows that sequencing findings of some HD positive samples.

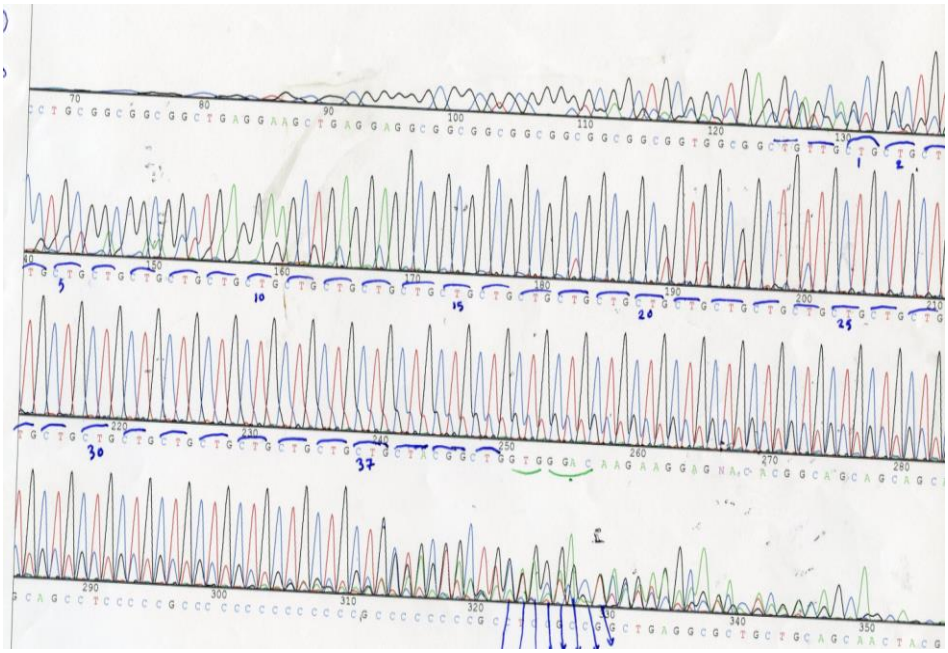

Figure 2. Electrograph of a region of the HD gene in a person with Huntington's disease.

Discussion

Medical practitioners faced with several diseases and disorders, which are hard to treat and even diagnose[20-23]. Huntington's disease is a dominant autosomal disorder is associated with progressive neurodegeneration that occurs in all races and is caused by mutations in the *HD* gene[24]. The present study was performed on 112 patients with suspected Huntington's disease. In these patients, movement disorders, mental disorders, dancing disorders, and etc. are observed. Of the 112 patients, 56 had Huntington's disease (50%) and another 56 (50%) had no increase in the number of replications in the *IT15* gene despite the Huntington-like clinical sign. In fact, other cases didn't have the *HD* gene. Among these patients with Huntington's disease, patients with a family history have been observed. A number of patients (with a family history) developed the disease at an early

age and had a pre-existing phenomenon. Studies have shown that when a mutated allele is inherited from father to child, the incidence of the disease is lower at an older age, and most patients who start the disease in adolescence and youth inherit their mutated allele from their father[25]. As in the present study, patients who started the disease in adolescence and youth inherited their mutated allele from the father. Three patients were also found to have increased CAG recurrence due to transmission of the mutated allele from father to child.

For example, a 7-year-old boy went to the lab with Huntington's disease. Family examinations revealed that his father and paternal grandfather were ill. The disease occurred in the father at the age of 26 and in his paternal grandfather at the age of 47. Transmission of the disease allele from father to child increases the amplitude of CAG replication (normal allele 14 replicates and pathogenic allele 101 replicates) in the child. Genealogy of this patient in Figure 1.

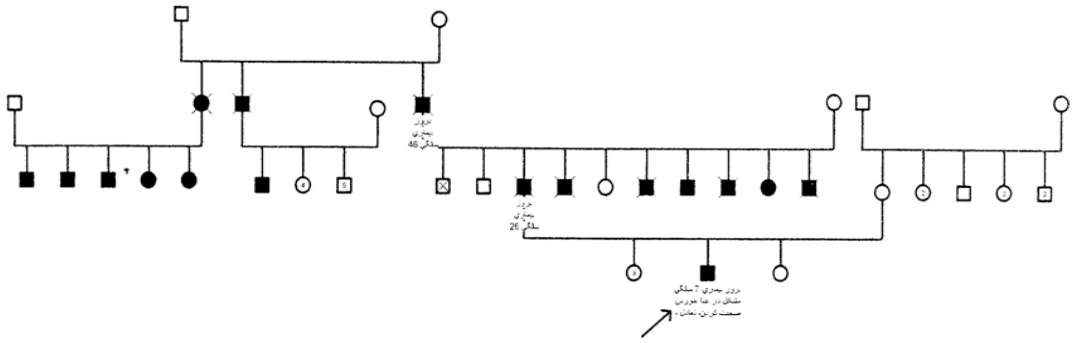

Figure 1. Genealogy of above-mentioned patient.

A number of patients have also been identified who do not have a family history in any of the family members, it can be said that these people have acquired the disease due to new mutations.

Another similar study was performed in Portugal on patients with the HDL phenotype (some of whom had a family history) and examined the *JPH3*, *PRNP*, and *TBP* genes. These patients were divided into 3 groups in terms of clinical symptoms. The first group had only movement disorders, the second group had only mental disorders and the third group had both mental and motor disorders. All patients were normal for JPH3 and TBP genes. Among these patients, only one member of a family had a mutation in the PRNP gene[26].

Conclusion

In conclusion, 112 patients were examined for the *HD* gene presence and 56 cases were positive and were recognized as definitely positive for the Huntington's disease. It seems that *HD* gene detection is an applied method to identify the Huntington's disease among patients with the clinical signs.

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