

# The Study of the Recurrent Sequence of CAG in Three Generations of an Iranian Family Suffering from Huntington Disease via PCR-Sequencing

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**Abstract:** *Background: Huntington's disease (HD) is the most common neurodegenerative one having the dominant autosomal inheritance which is being characterized by the excessive uncontrolled motor movements and the deficits in the emotional and cognitive functions. The mutation responsible for Huntington disease leads polyglutamine protein to be distributed in Huntington protein which carries more toxic functions towards Huntington protein. The aim of the study is to design a PCR system to diagnose Huntington disease which can detect the genotype of the disease.*

**Index Terms:** *Huntington, PCR, CAG sequence*

## I. INTRODUCTION

Huntington disease (HD) is the one of advancing disease accompanied by destroying the nerves. It is usually occurred in middle aged people. The disease causes Corei mutation. It is often along with the mental changes. The symptoms of the disease are as follows: Mental disorder, sense of dancing, the decrease in the function of perception and the wisdom decline (Kremer Bet et al. 2002). The prediction of the HD points to a strange phenomenon through which the mutated HTT allele heritage through the gamete of a male person indicates the clinical symptoms compared with the mutated HTT allele heritage through the gamete of a female one. Generally speaking, the infants experience HD symptoms for 8 years sooner than their fathers (Ranen et al. 1995). In these infants, the CAG distribution has been expanded more (Telenius et al. 1995; Telenius et al. 1994; Mac Donald et al. 1993). The HD heritage is of the dominant autosomal and is related to the expansion of CAG repetitions (Hayden MR et al. 1981; Zuhlke C et al. 1993; Novelleto A et al. 1994). 36 to 39-number repetitions of CAG can cause the onset of the disease. In more advanced stage, the 40-number repetitions and more of CAG can cause the disease to be completed. It is determined that most of the people who possess 60 repetitions manifest HD symptoms before their maturity age. It is called JHD (J=Juvenile) (Zuhlke C et al. 1993). The gene IT-15 possesses 67 exons. Its length is 200kb. The coded protein Huntington consists of 3136 Amino Acids. It weighs 350KD. Investigating the total genome, it is determined that the mutation of the CAG expansion agent happens in exon 1.

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## II. MATERIALS AND METHODS

In this study, three generations of the members of a 47-people Iranian family with HD symptom manifestations in one of the members of the family and also 47 samples of the healthy people without HD background were studied and investigated.

The criterion of the study is all the members of the family who have manifested the clinical manifestations of HD in one of the members of the family. First, 2cc of blood sample was poured into CBC tubes containing anticoagulant material called EDTA and kept in -20°C temperature. After sampling the genome DNA of all people, all samples were analyzed by PCR and PAGE and then coloring silver nitrate. In this study, sampling DNA of the subjects was done by the commercial kit called Geno Plus mini in Viogene Company in Germany. The samples were investigated by PCR-Sequencing.

The PCR thermal cycle consists of the elementary denaturation in 95°C during 10 minutes and the second cycle consist of three stages with 35 repetitions. The secondary denaturation was done in 95°C during 40 seconds. The connection of primer was done was done in 57°C during 30 seconds. The lengthening stage was fulfilled in 72°C during one minute and; eventually, the final lengthening was done in 72°C during 5 minutes.

The reaction compounds consist of the following ones: 25mmol/L magnesium chloride (MgCl<sub>2</sub>), 10mmol/L Deoxyribonucleotide triphosphate (dNTPs), 10 pmol/L primer Mix, 5 U/L Hot Start Taq Polymerase, 300 ng DNA, 10 µl DMSO, 50 mm NaCl, 50 mm KCl. The PCR reaction was done in the final volume of 25 µL.

After confirming the correctness of doing PCR reaction and observing PCR products on 1% Agarose gel, the PCR products in 8% Polyacrylamide gel become Electrophoresis and coloring silver nitrate was executed (figure 2). Also, the PCR product succession was determined for determining the repetition after PCR reaction (figure 3). After investigating the repetition position, couple of primers containing suitable CG designed by Gene Runner software (figure 1).

## III. RESULTS

Merely five out of 47 members of the family (23.5%) were suffered from the disease (tablet 1). The number of CAG repetitions was 39 in ill people. The disease contagion in 2 people of the family (9.4%) has been paternal and in 1 person (4.7%) has been maternal. Because two people of the family members weren't aware of their fathers and mothers' disease,

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it wasn't determined that the disease contagion was paternal or maternal. So far, four people of the subjects having disease allele did not manifest any symptoms till the age of 90, 38, 50 and 57. Also, one of the subjects has manifested the HD symptoms when it was nearly 70. There has not been any relative marriage among the subjects. 47 subjects (all 95 allele) possessed CAG natural repetitions between 10 to 35 ones. The ill people have been identified in the family tree of the family (figure 4).

### IV. DISCUSSION

The result of our study shows that the designed single PCR can correctly duplicate the sequence of the mentioned repetition in specialized manner. Also common determining the sequence of the duplicated reaction product can easily indicate the number of correct repetition.

The results of our study show that five patients among which one the HD symptoms has been manifested possess CAG sequence expansions in investigated family. The natural relatives are in lack of CAG expansion existing in HD or IT15 in short chromosome arm 4.

According to the pattern of family tree in dominant autosomal (AD) diseases, existing just one mutant allele in one of the parents is enough for inheriting the disease. In AD diseases in standard case, the suffered person has a suffered father or mother. However, in the genes related to trinucleotide disorders such as HD particularly with the paternal origin, the disease occurrence depends on the extent of the expansion of repetitions and the manifestation of the clinical symptoms (Walker FO. 2007; Nance MA & Myers RH. 2001).

Bozza A and associates (Bozza A et al. 1995) observed a patient, in their studies, who was 74 bearing HD symptoms well; while, he was in lack of family background. In our study, one of the patient's father was in lack of HD symptoms while he was nearly 90 (II – 5 person).

Our result in accordance with some similar reports which prove that the HD existence is due to the existence of CAG repetitions in HD gene (Boneli RM & Wenning GK. 2006; Walker FO. 2007; Nance MA & Myers RH. 2001; Djoussé L et al. 2003).

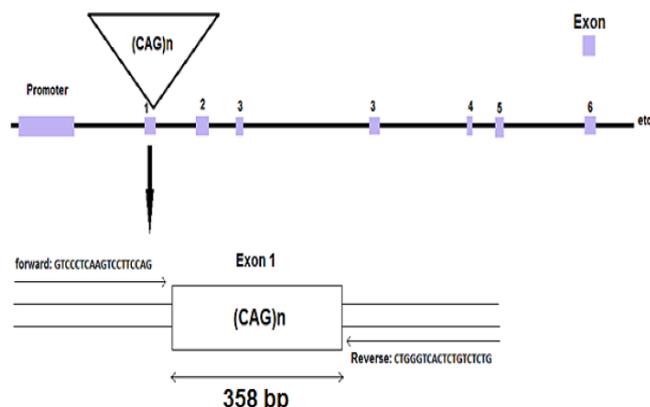
There is a meaningful relationship between the onset age of the disease and the relationship length. The more the length of repetition, the less the onset age of the disease. Nearly 50% of variety in the number of sequence in the disease onset age is

observed (Pour-Jafari et al. 2009). In healthy people, the number of repetitions is between 15 and 30 and in ill ones the number is 40 or more.

The HD symptoms usually occur and appear at the middle of life; but in 7% of cases, 21-year-old people expose these symptoms (Pour-Jafari ET al.2009).

In our study, the patients have not exposed the symptom when they are young. A 72-year old patient exposed HD symptoms as a case. Wojaczyńska-Stanek K and associates have revealed that in the case of the gene inherited by the father, the magnitude of sequence usually more than 60 repetitions and the individualistic clinical fathers of the person are of the factors that cause HD exposure in youth (Wojaczyńska-Stanek K et al. 2006).

In brief, the present study has posed PCR-Sequencing to investigate CAG repetitions in HD gene as a suitable technique which provides the possibility to diagnose HD existence in patients. Finding sequence for the gene mHTT could determine the investigation veracity.

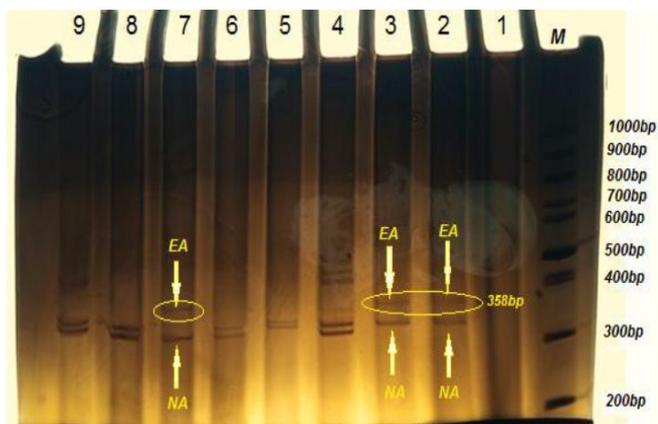


**Figure 1 Is The Schematic Figure of HD Gene Along with Primers Used for a Part of Exon 1.**

In the above figure, a part of HD gene has been shown containing the position of CAG repetitions and the position of primers used for reproducing and increasing a part of gene containing CAG repetitions.

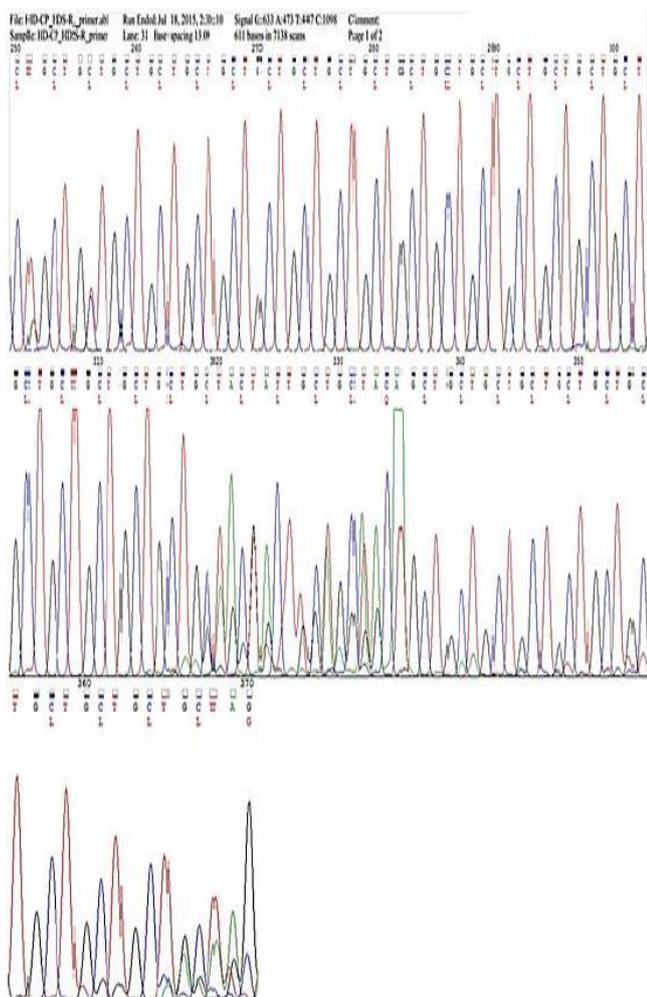
**Table 1. The Characteristics of The Patients Suffered From Huntington Disease Which Has Been Diagnose by The Molecular Methods.**

Family NO.	Gender	Age	Repeat NO.	Children NO.	Patient Children
Family 1	Female With 4 years disease history	74	39 & 20	2	One son with 39 repeats
Family 2	Male	90	39 & 22	5	Two sons with 39 repeats
Family 3	Male	38	39 & 19	0	
Family 4	Male	57	39 & 23	2	One son and daughter with normal allele
Family 5	Male	50	39 & 22	0	



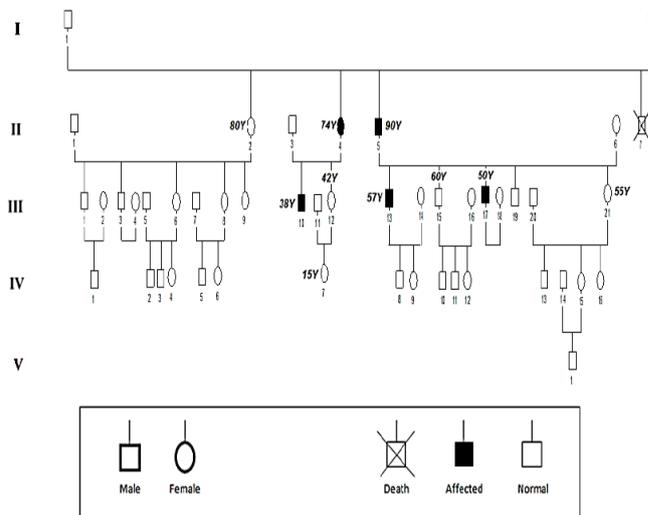
**Figure 2. PCR Product Analysis in Polyacrilamide Gel.**

Line M: 100 bp DNA marker, line 1: the negative control sample, line 2: family 1, the patient with 39 and 20 repetitions, line 3: family 3, the patient with 39 and 19 repetitions, line 7: family 4, the patient with 39 and 23 repetitions, lines 4, 6, 5 and 8: the healthy people, line 9: the healthy and evidence sample.



**Figure 3. Finding Sequence in HD Gene in One of the Members of the Family.**

In the above figure, one the sequenced samples which is related to one of the passions is observable. The number of repetitions has been distinct by L letter (red). The sequence has been shown by CTG because the HD gene is in the form of Heterozygote.



**Figure 4. The pattern of the studied family tree (the Roman numbers show the generation arrangement. The numbers show the people numbers in each generation. The number before the letter Y indicates each person's age).**

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### REFERENCES

1. Bonelli RM, Wenning GK.(2006). Pharmacological management of Huntington's disease: an evidence-based review. *Curr Pharm Des*; 12: 2701-2720.
2. Bozza A, Malagù S, Calzolari E, Novelletto A, Pavoni M, del Senno L.(1995). Expansion of a (CAG)*n* repeat region in a sporadic case of HD. *Acta Neurol Scand*; 92: 132-134.
3. Djoussé L, Knowlton B, Hayden M, Almqvist EW, Brinkman R, Ross C, et al . (2003). Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. *Am J Med Genet A*; 119: 279-282.
4. Hayden MR, Bruyn G.(1981) Huntington's Chorea. New York: Springer Verlag; 1-13.
5. Zuhlke C, Riess O, Schroder K, et al.(1993) Expansion of the (CAG)*n* repeat causing Huntington's disease in 352 patients of German origin. *Hum Mol Genet*; 2(9): 1467-9.
6. Mazdeh M, Pour-Jafari H, Ghaleiha A, Pour-Jafari B (2008) A survey of (CAG)*n* repeats causing juvenile Huntington disease in an Iranian family with 4 affected members. *Neurosciences*; 14 (3): 273-276
7. Nance MA, Myers RH. (2001). Juvenile onset Huntington's disease—Clinical and research perspectives. *Ment Retard Dev Disabil Res Rev*; 7: 153–157.
8. Novelletto A, Persichetti F, Sabbadini G, et al.(1994). Analysis of the trinucleotide repeat expansion in Italian families affected with Huntington disease. *Hum Mol Genet*; 3(1): 93-8.
9. Walker FO.(2007). Huntington's disease. *Lancet*; 369: 218-228.
10. Wojaczyńska-Stanek K, Adamek D, Marszał E, HoffmanZacharska D.(2006). Huntington disease in a 9-year-old boy: clinical course and neuropathology examination. *J Child Neurol*; 21: 1068-1073.