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Molecular analysis and prevalence of Huntington disease in northwestern Iran

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Background/aim: Huntington disease (HD) is a progressive adult-onset neurodegenerative disorder presenting an autosomal dominant inheritance. Since there is no information on the prevalence of HD in northwestern Iran, the aim of the present study was to determine the prevalence of HD and the number of CAG trinucleotide repeats in the population of northwestern Iran.

Materials and methods: Genomic DNA was extracted from whole blood by the salting-out method. DNA samples were analyzed to determine the number of CAG trinucleotide repeats of the HD gene. An abnormally large number of CAG repeats, which is a diagnostic factor for the disease, was detected by polymerase chain reaction and agarose gel electrophoresis.

Results: Out of 40 cases, we identified 14 nonkindred individuals with one expanded CAG allele at the IT15 gene. The frequency of the HD mutation in our group of patients was 35%. Expanded alleles varied from 36 to 70 CAG repeats, and normal alleles in HD patients varied from 20 to 26 CAG units.

Conclusion: We found a significant correlation between age at onset of the disease and length of the expanded CAG tract: the lower the age, the longer the trinucleotide repeats length.

Key words: CAG trinucleotide repeats, Huntington disease, IT15

1. Introduction

Huntington disease (HD) is a progressive hereditary neurodegenerative disorder that affects the central nervous system (1). HD is an autosomal dominant disease due to the expansion of CAG trinucleotide repeats in the HD gene (IT15) located at 4p16.3 and encoding a protein called huntingtin (2,3). The number of the repeats is normally between 6 and 34 in healthy individuals, while it is expanded to more than 40 repeats in people with HD (4,5). The expansion of CAG repeats results in an expanded polyglutamine sequence in the resulting protein, which leads to abnormal interactions of the huntingtin protein with other nuclear proteins. These abnormal aggregations in brain cells cause cellular death (6). In HD, the basal ganglia and the cerebral cortex, which control concentration, memory, and coordination of movements, are affected more than the other regions (7). The exact function of the huntingtin protein is unclear, but it is known to be associated with microtubules and synaptic vesicles, suggesting a role in the transport of substances or cell components along the microtubules to the synapses (the structures that facilitate communication between neurons) (8,9). In addition, there is some evidence that apoptosis plays a role in HD (10,11).

One of the major characteristics of HD is uncontrollable muscular movements, or chorea, and hence the name Huntington's chorea of the disease (12). The other characteristics of the disease are short-term memory abnormalities, poor concentration, speaking problems, and cognitive (perception, awareness, thinking, and judgment) and psychological issues, such as mood swings, clumsiness, changes in gait, fidgety movements, irritability, depression, and uncharacteristic aggressive behavior, which can be quite different even among members of a single family (13,14). In addition, the rare phenomenon of “anticipation” has been observed in HD, which means that the age of onset and the severity of symptoms will decrease and increase, respectively, in subsequent generations (6,15). The anticipation phenomenon is believed to be the result of instability of the trinucleotide repeats during meiosis (16).

Although the symptoms are usually detectable in the fourth decade of life, the age of onset is highly variable, in that symptoms are observed at early ages or in people as old as 90 years (17). The average life expectancy is almost 15 years after the onset of symptoms (18,19). There is no complete therapy for HD, but in the case of an early
diagnosis, one might take advantage of therapeutics that alleviate the symptoms and control the progression of the disease. Early symptoms can be managed and alleviated with drugs, such as depleting agents and other forms of therapy, to improve the quality of life. In addition, chorea can be treated with dopamine receptor blocking drugs (20).

Based on comprehensive studies in different populations, the prevalence of the disease is estimated to be 5 in 100,000 (18,19,21,22). However, there is no statistical information on its prevalence in northwestern Iran. Therefore, the aim of the present study was to estimate the prevalence of the disease in the area and to determine the number of CAG trinucleotide repeats in HD alleles.

2. Materials and methods

2.1. Subjects

A group of 42 clinically affected and unrelated individuals, including 18 females and 24 males, were recruited. Blood samples were obtained from individuals for DNA testing after obtaining written informed consent. According to their clinical details, which were based on extensive records documenting their neurological examination, all patients showed typical HD symptoms such as chorea, movement impairment, cognitive disorders, and personality changes.

The probability of developing other neurodegenerative diseases was ruled out by pedigree analysis and clinical tests in all cases.

A group of 21 unaffected individuals was recruited as controls that were similar to the clinical cases age- and sex-wise. The patients were referred to the outpatient clinic of Tabriz University of Medical Sciences between June 2012 and April 2015, and a 5-mL blood sample was drawn from all the subjects. This study was approved by the ethical committee of Tabriz University of Medical Sciences (Tabriz, Iran).

2.2. DNA extraction

The salting-out method was used to extract genomic DNA from the peripheral blood of subjects and controls. To determine the DNA concentration and quality, extracted DNAs were analyzed by NanoDrop.

2.3. Polymerase chain reaction (PCR)

The basis of screening tests is to measure the sizes of the CAG repeats in each individual by performing PCR (23). The PCR amplification method was conducted for the IT15 gene in the Bio-Rad T100 thermal cycler. PCR amplification of the CAG repeat in the IT15 gene was performed with primers HD1 (5′-ATGAAGGcCTCCGAGTCCCTCAAGTCCCTCC-3′, Tm: 75 °C) and HD3 (5′-GGCGGTGGCGGCTGCTGCTGC-3′, Tm: 79 °C) (2,23,24). The primer pair was designed by using a software tool from the NCBI database, available in the primer BLAST tool section.

To perform PCR, 7.5 µL of mix red from Cinnagen Company, 1 µL of forward primer, 1 µL of reverse primer, 5 µL of Q-Solution, 1.5 µL of H2O, and 1 µL of DNA were added to microtubes for a final volume of 17 µL.

The thermal profile for PCR was as follows: 98 °C for 15 min; 35 cycles of 98 °C for 1 min, 70 °C for 1 min, and 72 °C for 2 min; and 72 °C for 10 min as final extension.

2.4. Electrophoresis

The stained PCR products were used for electrophoresis on a 3% agarose gel containing Safe Stain. The sizes of visualized bands of the IT15 gene in healthy controls and HD patients were 100–120 and 200–250 base pairs, respectively. The number of CAG trinucleotide repeats in respective chromosomes of HD patients was estimated using the DNA fragment size determined by electrophoresis (Figure).

3. Results

The number of CAG repeats within the gene determines whether an individual has normal (5–26 repeats), normal with paternal meiotic instability (27–35 repeats), alleles with reduced penetrance (36–39 repeats), or HD-affected (>40 repeats) alleles. However, normal results do not guarantee a definite exclusion of developing HD, as there is still a probability that other very rare mutations may produce the HD phenotype.

In this study, 42 patients were examined and 15 affected individuals were diagnosed with heterozygous expanded CAG trinucleotide repeats in the IT15 gene. The number of trinucleotide repeats within a normal allele is approximately 20 to 26, whereas in mutant alleles it is approximately 40 to 70 (Table).

Figure. Analysis of PCR products containing the expanded CAG repeat at the IT15 gene. PCR products were analyzed on 3% agarose gel. Allele sizes were determined by comparison with a sequencing ladder (50 bp). 1- Ladder; 2- no DNA; 3- positive control; 4- patient; and 5- normal subject.
The youngest affected individual was a 35-year-old patient with the most number of CAG trinucleotide repeats (70 repeats), which resulted in more severe symptoms, and the oldest affected individual was a 70-year-old patient with the least number of CAG trinucleotide repeats (40 repeats) within one of the mutant HD alleles.

The number of CAG trinucleotide repeats in affected individuals who were between 40 and 60 years old when symptoms emerged was 45–60. Therefore, the higher the number of repeats, the lower the age at the onset of symptoms and the more severe the symptoms.

Autosomal dominant inheritance could be documented in all but 2 patients who had no family history of the disease. The transmission of the disease was paternal in 10 and maternal in 3 cases. There was no significant difference in the size of expanded CAG repeats between patients with paternal inheritance.

**4. Discussion**

In recent decades, a mechanism of mutation has been reported in humans, called dynamic mutation, which is the result of the expansion of trinucleotide repeats (25). HD, along with other neurodegenerative diseases such as Kennedy disease and spinocerebellar ataxias (1–3,6,7), is the result of CAG trinucleotide expansion in the corresponding gene, which results in polyglutamine tracts in the resulting protein (26,27).

In the present study, the expansion of CAG trinucleotides has been detected in 36% (15/42) of HD-suspected individuals. This is the first molecular study of the disease in northwestern Iran. The results indicate a significant relationship between the length of the repeats and the age of onset. The length of CAG repeats in healthy individuals and the normal allele of the affected ones was 20–26, while most of the patients between the ages of 41 and 60 had nearly 50 repeats in at least one allele. The average lengths of CAG trinucleotide repeats in normal and HD alleles observed in this study are comparable to the findings of other studies (24,28). It should be mentioned that, using gel electrophoresis, it is only possible to determine the approximate number of repeats; to determine the exact number, sequencing of the exons of the gene is essential.

There is some evidence that shows that de novo mutations can be the cause of sporadic HD (24,28). In the population we studied, there were 2 (13%) individuals without a family history of HD.

The results showed that the expansion of CAG in consecutive generations occurs only when the mutant allele is inherited paternally (24,28). Therefore,
meiotic instability of the HD allele takes place during spermatogenesis (29). In the present study, by analyzing the pedigrees of patients, it was observed that 66% (10/15) of the individuals inherited the mutant allele paternally and 20% (3/15) inherited it maternally. In addition, in paternal inheritance, the patients had more severe symptoms at earlier ages as compared to their fathers.

Regarding HD diagnosis, it is important to not misdiagnose the disease as other similar diseases, such as chorea gravidarum, hyperthyroid chorea, and vascular hemichorea, all of which have chorea-like symptoms. Unlike HD, these diseases are not hereditary and do not involve permanent speech and cognitive disorders (25).

In recent years, several diseases with symptoms similar to HD have been identified. These are referred to as HD-like (HDL) diseases. HD includes approximately 90% of hereditary chorea cases, while HDL includes only 1% (6,15). Phenotypic overlap between HD and HDL is a major challenge for HD diagnosis. There are several mutations in various genes that bring about HDL diseases, such as HDL1, HDL2, and HDL4, all of which have an autosomal dominant inheritance. HDL3 is probably inherited in an autosomal recessive pattern, which means that both copies of the gene in each cell have mutations (8,9,18).

In the population that we studied, those who did not have HD mutations, i.e. CAG trinucleotide expansion, probably had HDL mutations. Therefore, it is recommended that along with HD mutations, researchers evaluate common HDL mutations.

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References


