A survey of (CAG)n repeats causing juvenile Huntington disease in an Iranian family with 4 affected members

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ABSTRACT

ينتج مرض هنتنغتن بواسطة التمدد المتكرر لمثلث النوكليوتيد (CAG) في الجين المرمز لهنتنغتن (Htt) وهو أحد الأمراض المتعددة الأحماض الأمينية. تظهر أعراضه البدنية على مدى كبير من الأعمار، وتظهر بشكل رئيسي لدى الأشخاص في أواخر الأربعينات/ أوائل الخمسينات. تشير معظم المراجع إلى أن العمر إذا كان أقل من العشرينات فإن ذلك يعرف بمرض هنتنغتن الصبياني. نستعرض في هذا التقرير حالتنا لدى عائلة إيرانية لديها أربعة أشقاء وشقيقات مصابين (أختين وأخوين)، بالإضافة لهؤلاء الأطفال الأربعة المصابين، لدى العائلة خمسة ذكور طبيعيين. ليس لدى تاريخ العائلة أي حالات أخرى. كان عمر نوبة المرض لدى العائلة 20 إلى 25 عام. لم يكن الوالدين مصابين، كما أنهما لم يكونا أقارب أو أبناء عم من الدرجة الأولى. اظهر التحليل المرضي لعامل مرض هنتنغتن (CAG) للأعضاء الأربعة المصابين وجود توسع حليلي مع 46، 50، 46 و 44 تكرارٍ في الأشقاء الأربعة. أشارت نتائجنا إلى أن عمر عشرون عاماً قد لا يكون نقطة محدودة الاستقرار لجميع الحالات لمرض هنتنغتن الصبياني وربما تكون أعمار النوبات مرتبطة مع حجم (CAG) المتكرر في مثل هؤلاء الأفراد.

Huntington's disease is caused by a trinucleotide repeat expansion (CAG)n in the gene coding for Huntingtin (Htt) and is one of the several polyglutamine diseases. Its physical symptoms occur in a large range of ages, with a mean occurrence in a person's late 40's and early 50's. Almost all references indicated that if the age of onset is below 20 years then it is known as juvenile HD. Our case was an Iranian family with 4 affected siblings (2 sisters and 2 brothers). In addition to 4 affected children, they had 5 normal male progenies. There was no any other case in their family history. The onset age of the disease in our case family was 20 to 25 years. Their parents were unaffected and nonconsanguineous. Analysis of the pathogenic (CAG)n repeat region of the HD gene for the affected members have showed an expansion allele with 46, 50, 46, and 44 repeats in 4 affected siblings. Our results indicated that the age of 20 years maybe is not a stable limit point for all cases of juvenile HD, and perhaps onset ages are related with the CAG repeat sizes in such individuals.

Neurosciences 2009; Vol. 14 (3): 273-276

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Received 5th November 2008. Accepted 2nd February 2009.

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Huntington's disease (HD) is a neurodegenerative condition that leads to neuronal loss in the striatum and cortex, and to the appearance of neuronal intranuclear inclusions of Huntingtin (Htt) protein.[1] The exact mechanism of HD is unproven and there is currently no proven cure, so symptoms are managed with a range of medications to treat individual symptoms and supportive services.[2] Global incidence varies, from 3-7 per 100,000 people of Western European descent, down to one per 1,000,000 of Asian and African descent.[3] The onset of physical symptoms in HD occurs in a large range around a mean of a person's late 40's to early 50's. If symptoms become noticeable before a person is 20, their condition is known as juvenile HD.[4] The genetic defect causing HD, with an autosomal dominant (AD) mode of inheritance, has been identified as an unstable expansion of a trinucleotide (CAG) repeat sequence within the coding region of the interesting transcript 15 (IT15) gene on chromosome 4.[1] Different studies have shown that the length of CAG repeats, in most cases, has

Disclosure. This study was funded by the research deputy of Hamadan University of Medical Sciences, Hamadan, Iran.

a strong inverse correlation with the age at onset of HD,[5] but some others claimed that there is no such relationship between the number of CAG repeats and the rate of clinical decline.[1] These findings suggest that the CAG repeat length may influence or trigger the onset of HD, but other genetic, neurobiological, or environmental factors contribute to the progression of illness and the underlying pace of neuronal degeneration.[1] In the present study, we have reported an Iranian rural family with 4 affected siblings in whom the expansion of the CAG repeats were not as large as most reports, but it is accompanied with severe clinical manifestation and juvenile onset age, but not below the age of 20 years. The present report is a sample showing diversity in the size of the CAG repeats and the onset age of HD in this area.

Case Report. Our case family at the time of referral consisted of a 60-year old widow with 2 affected sons, 2 affected daughters, 5 normal sons (3 alive plus 2 deceased). Her husband passed away at the age of 70 years, 5 years ago. She said that her husband was not affected with the same illness. The mother (II-7) was normal with a nonconsanguinity marriage. She also mentioned, her 2 deceased sons were phenotypically normal and the cause of their death was an accident, their age was around 20. Continuing, they have no any similar affected individual in their family history.

Figure 1 shows the pedigree of considered family. Ages at onset were based on information provided by the patients and their mother. The clinical diagnoses of HD were made locally by a neurologist and geneticist. In addition, clinical details on the patients were derived from extensive study of records, documented neurologic examinations, and results of preclinical tests (hormones, MRI, and ceruloplasmin) that were considered.

After obtaining consent from all 4 patients (III-5, III-7, III-9 and III-10), their mother and youngest brother (III-14), blood samples were obtained with ethylene diamine tetra acetic (EDTA) acid. Deoxyribonucleic acid (DNA) extraction was carried out using the salting out method. The length of the CAG trinucleotide repeat was determined using 35 cycles of the polymerase chain reaction (PCR) to amplify the considered trinucleotide. After an initial denaturation of 2 minutes at 96°C, there were 12 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 2 minutes, followed by 23 cycles at 92°C for 30 seconds, 65°C for 30 seconds, and 72°C for 2 minutes; final extension was at 72°C for 10 minutes. The PCR was carried out in a final volume of 25 µL with the primers HD1 (5'-ATGAAGGGGYFG GAGTCGGTGAAGTGG'VITG-3') and HD3 (5'-GGGGTGGGGGGTGTTGGTGGTGGTGGTGC-3'). Reaction mixtures contained 2 mmol/L magnesium chloride ($MgCl_2$), 16.6 mmol/L ammonium sulphate $(NH4)_2S0_4$, 67 mmol/L hydroxymethyl-hydrochloric

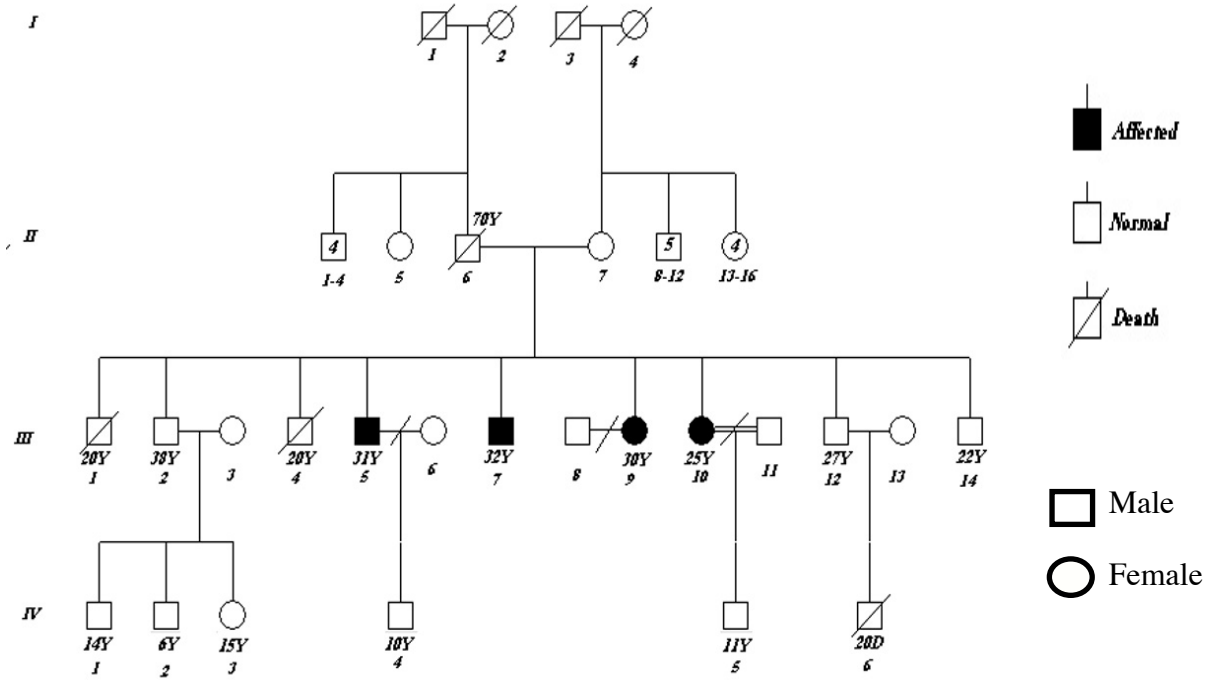

Figure 1 - Pedigree pattern of the case family (Roman numbers indicate the order of generations; Arabic numbers indicate the order of individuals in each generation; numbers before letter "Y" - year or "D" - day, indicate age of individual at the time of interview or the age at death for deceased individual).

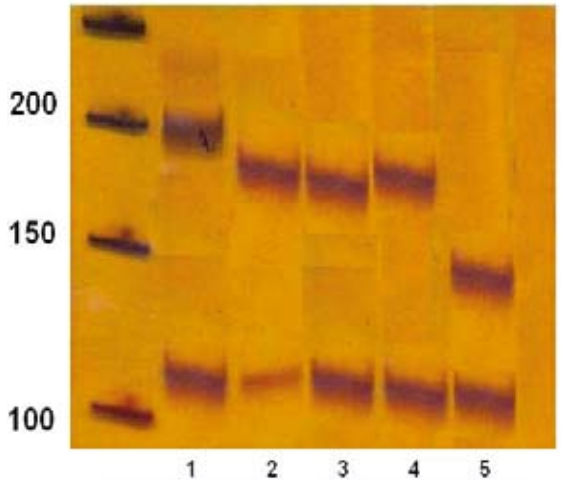

Figure 2 - Polymerase chain reaction (PCR) analysis in 10% polyacrylamide gel of trinucleotide repeats in 4 affected sibs (lanes 1-4), and one of their normal brothers (lane 5) as control. The lane before number 1 is DNA molecular marker.

acid (Tris-HCl), pH 8.8, 67 µmol/L Disodium ethylenediamine tetraacetate (Na_2EDTA), 35 ml/L formamide, 10 mmol/L ß-mercaptoethanol, 2.5 µmol/L bovine serum albumin, 200 mmol/L of each Deoxyribonucleotide triphosphate (dNTP) (with a final ratio of 1:3 Deoxyguanosine triphosphate (dGTP): 7-Deazaguanosine-5'-Triphosphate (7-deaza-GTP), 12.5 pmol each of HD1 and HD3, 1.25 U of Taq polymerase, and 250-500 ng of genomic DNA. From each amplified DNA sample, 5µL was tested on 8% acryl amid gel with Tris-acetate-EDTA buffer. After electrophoresis, the DNA was visible with silver staining (**Figure 2**).

Discussion. The results of our work showed that all 4 patients, with clinical evidence of HD, in the considered family had expanded CAG repeats. Their clinically normal siblings had no expansion in the HD gene, or IT15 on the short arm of chromosome 4. The pedigree pattern of the family showed an AD, needing only one affected allele from either parent to inherit the disease. Although this generally means there is a one in 2 chance of inheriting the disorder from an affected parent, the inheritance of HD and other trinucleotide repeat disorders is more complex. In the standard mode of AD, the affected individual usually has an affected parent, but in trinucleotide repeat disorders mutant genes, especially with paternal origin, the expansion of repeats tend to expand and manifest clinically.[3,4] Bozza et al[6] explained a (CAG)n expansion in a 72-year-old woman with typical HD symptoms, but no family history of the disorder. In our work, the father of the patients (II-6) was dead, and had no HD based on the results of our interview with his family.

Our results agree with some similar reports, in which the clinical manifestation of HD was known as a consequence of CAG expansion in HD gene.[2-5] On the other hand, the difference in our work with those results is that in our cases the expansion of the mentioned gene was not as large as theirs. Although in at least one work, researchers have reported some opposite results in which in several cases with HD there was no CAG expansion.[7] They showed that the CAG trinucleotide expansion is the molecular basis of HD worldwide, and is a highly sensitive and specific marker for inheritance of the disease mutation. The calculations of sensitivity and specificity were based on the study of 995 patients with clinically evident HD, and expanded CAG repeats; 12 people with clinical signs and symptoms compatible with HD, but normal CAG repeats; and those with another neuropsychiatric disease who all had CAG repeats within the normal range (113 patients). They reported that their analysis yielded an estimated sensitivity of 98.8% (95% confidence interval, 97.7-99.4%), and a specificity of 100% (95% confidence interval, 95.2-100%) for the use of the number of CAG repeats to identify those with the mutation for HD.

There is a highly significant correlation between the age of onset and the repeat length, which accounts for approximately 50% of the variation in the age of onset.[8] In the present the work, the results indicated the same results. The numbers of CAG repeats in our patients were 46, 50, 46, and 44, (mean = 46.5) and the number of the repeats in their clinically normal brother was less than 36 repeats. The considered gene normally has 15 to 30 repeats and an expansion to 40 or more is associated with HD. Huntington's disease usually has a mid-life onset, but a juvenile form, defined by onset of symptoms before the age of 21 years, is present in approximately 7% of HD cases.[4] In our patients the clinical manifestation started at the ages 20 to 25 in 4 affected siblings. Wojaczyńska-Stanek et al stated that Juvenile HD is characterized by (A) transmission from an HD affected father, (B) an unusually large repeat size, usually of 60 or more units, and (C) unique clinical features, including rigidity and seizure disorder.[9] A group of researchers have reported a boy with juvenile HD that the number of CAG repeats were 95, the clinical manifestation started at the age of 3 and finally he died at the age of 11. We could not find the origin of the mutant gene, as the father was deceased and the mother did not agree to be involved in the study at the time of interview. Also, we are not sure of the situation of those 2 siblings that passed away before our study. Their mother has claimed that they were not affected and died by accident. In a work on a large number of affected individuals with different ethnic and national groups in Canada, the results showed that there were no

significant differences among national and ethnic groups in the number of repeats.[6]

In conclusion, our results showed that the age of 20 may not be a stable limit point for all cases of juvenile HD, and perhaps onset age is related with the length of gene expansion.

Acknowledgment. *The authors wish to sincerely thank Dr. M. Houshmand, Dr. M. Rostami, and Dr. F. Talebzadeh for their useful help.*

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CASE REPORTS

Case reports will only be considered for unusual topics that add something new to the literature. All Case Reports should include at least one figure. Written informed consent for publication must accompany any photograph in which the subject can be identified. Figures should be submitted with a 300 dpi resolution when submitting electronically or printed on high-contrast glossy paper when submitting print copies. The abstract should be unstructured, and the introductory section should always include the objective and reason why the author is presenting this particular case. References should be up to date, preferably not exceeding 15.