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Intergeneration CAG expansion in a Wuhan juvenile-onset Huntington disease family

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Abstract: Objective To make early diagnosis of *IT15* gene mutation in a Wuhan juvenile-onset Huntington disease (HD) family, for providing them with genetic counseling, and making preparation for the further research on pathogenesis and experimental therapy of HD. **Methods** According to the principle of informed consent, we extracted genomic DNA from peripheral blood samples and carried genetic diagnosis of pathogenic exon 1 of *IT15* gene by modified touchdown PCR and DNA sequencing methods. **Results** Eight of twenty-five family members carried abnormal allele: III_{10}, III_{12}, III_{14}, IV_3, and V_2 carried $(CAG)_{48}$, IV_{11} and IV_{12} carried $(CAG)_{67}$, and IV_{14} carried $(CAG)_{63}$, in contrast with the 8-25 CAG trinucleotides in the members of control group. IV_{14} carried 15 more CAG trinucleotides than her father III_{10}. **Conclusion** The results definitely confirm the diagnosis of HD and indicate the CAG trinucleotide repeat expansion of *IT15* gene in this HD family. In addition, CAG expansion results in juvenile-onset and anticipation (characterized by earlier age of onset and increasing severity) of the patient IV_{12}.

Keywords: Huntington disease; early diagnosis; trinucleotide repeat expansion; genetic anticipation

1 Introduction

Huntington disease (HD) is an autosomal dominant disorder with characteristic of progressive and selective neural cell death, clinically associated with chorea, dementia, cognitive and affective impairment. HD has been reported in almost every population, but it is more common in Caucasians than in Asian and African American. In the United States of America, the incidence of juvenile HD is about 0.5–1.0 per 100 000 individuals, which is ten times lower than that of adult HD[1]. HD is correlated with increment of the CAG trinucleotide repeats in the important transcript 15 gene (*IT15*) called 'Huntingtin', and located on chromosome 4p16.3[2]. The length of CAG repeats can expand or contract during intergenerational transmission. The large expansion of CAG principally caused by paternal transmission tends to be responsible for juvenile onset cases[3]. Here we reported a Wuhan juvenile-onset HD family demonstrating the intergeneration CAG expansion.

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2 Materials and methods

2.1 Subjects The pedigree recruited from Wuhan of China contained four HD patients and other twenty-one family members (Fig. 1). Informed consent was obtained from all the subjects. Normal controls were obtained from the blood bank of Tongji Hospital, Huazhong University of Science and Technology. We stored all peripheral blood samples at –20 ºC, with one-tenth of volume of 2% EDTA as anticoagulant.

2.2 Genomic DNA purification Red blood cells were removed from the peripheral blood sample (500 μL) with cold

double distilled water. The pellets were digested with white blood cell lysis buffer (0.625% SDS, 0.1 mol/L NaCl, 0.05 mol/L Tris-HCl, and 0.001 mol/L EDTA) and protease K. Chloroform was employed to extract DNA. After washing with 70% ethanol, the purified genomic DNA was dissolved with double distilled water and then stored at –20 ºC.

2.3 PCR amplification According to the methods of Tang YP[4], two oligonucleotides (forward: 5'-CGA CCC TGG AAAAGC TGA TG-3' and reverse: 5'-GGC TGA GGA AGC TGA GGAG-3') were used to amplify the region containing instable CAG repeats in exon 1 of the *IT15* gene. The total 25 μL of PCR system contained 3 μL of 30 μg/mL genomic DNA, 4 μL of 2.5 mmol/L dNTP, 2 μL of 10 μmol/L primers, 12.5 μL of 2 × GC buffer (Takara, Japan) (or, 2.5 μL of 10 × buffer and 1.25 μL DMSO), 0.5 μL of 5 U/μL Taq DNA polymerase (Shanghai, China), and supplement with double distilled water. PCR was carried out under the following conditions: 95 ºC for 3 min, (95 ºC for 30 s, 68 ºC for 30 s, 72 ºC for 40 s) × 10 cycles, then (95 ºC for 30 s, 60 ºC for 30 s, 72 ºC for 40 s) × 30 cycles, and finally extending at 72 ºC for 3 min. According to the National Center for Biotechnology Information (NCBI), the PCR products should be about 163 bp and contain 21 CAG triplet repeats.

2.4 Agarose gel electrophoresis and DNA sequencing Five microlitres of amplified DNA products were analyzed by a 2.0% agarose gel electrophoresis, and stained with ethidium bromide (EB). Extract the PCR products with different molecular weight by TIANgel Midi Purification Kit (Tiangen Biotech, China). The purified PCR products were performed DNA sequencing by Shanghai Invitrogen Company.

3 Results

3.1 Case report III_{10}, III_{12}, III_{14}, and IV_{12} were HD patients with the classical symptoms, including choreic movements, decreased cognitive abilities, and psychological impairments. IV_{11} showed unsteady gait and limp, and IV_{14} demonstrated apparently frequent eye movement, which was suspected to be an early HD symptom. Currently, the other members

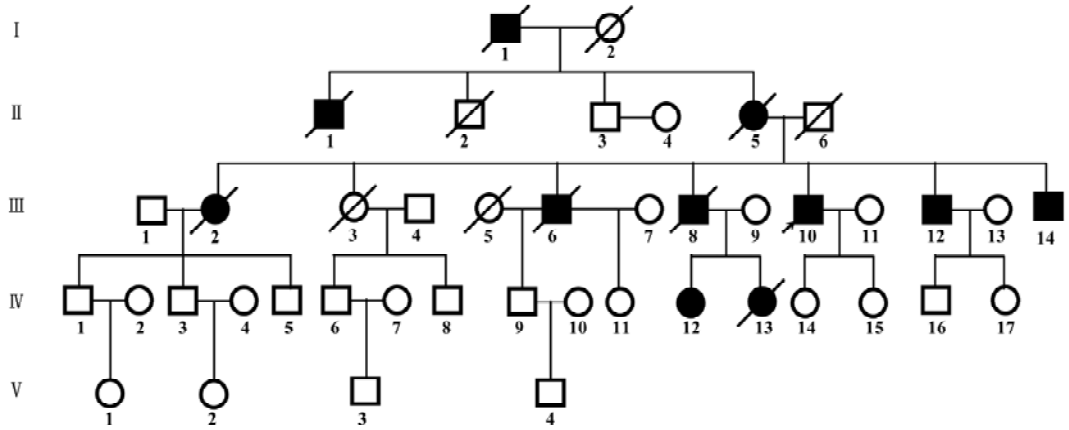

Fig. 1 Pedigree of the HD family. ■ male patient, □ healthy male; ● female patient, ○ healthy female; / died. ↗ the proband. III_{10}, III_{12}, III_{14}, IV_{12}: patients; IV_{11} and IV_{14}: possible patients; IV_3 and V_2: carriers with pathogenic gene.

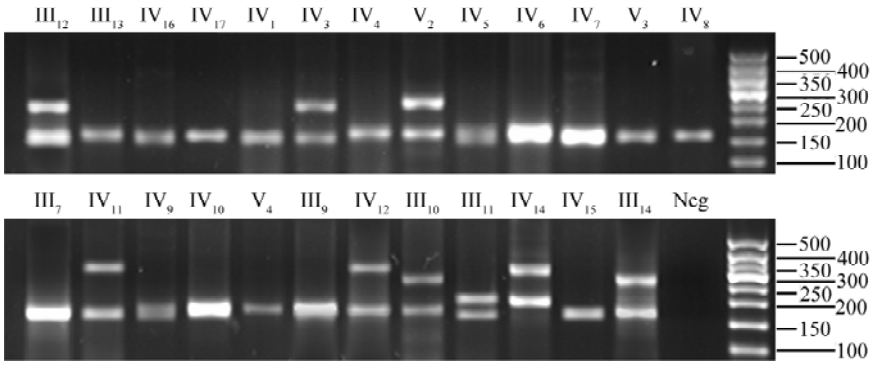

Fig. 2 Results of agarose gel electrophoresis of resulting PCR products of *IT15* gene. M: 50 bp DNA Ladder; III_{10}, III_{12}, III_{14} and IV_{12}: patients; IV_{11} and IV_{14}: possible patients with pathogenic gene; IV_3 and V_2: pathogenic gene carriers; the others are healthy members. Neg: negative control.

at risk of HD were phenotypically normal. Specifically to note, IV_{17} was an 18-year-old pregnant individual at risk of HD.

3.2 Analysis of the PCR products The electrophoresis on the 2.0% agarose gel showed that the normal bands ranged from 160 bp to 200 bp, while the pathogenic alleles varied from 240 bp to 300 bp (Fig. 2). Among all the family members, III_{10}, III_{12}, III_{14}, IV_3, IV_{11}, IV_{12}, IV_{14}, and V_2 carried the abnormal alleles of more than 240 bp. Pathogenic *IT15* gene was not found in all the other individuals at risk, including the pregnant female member.

3.3 DNA sequencing The results of DNA sequencing suggested that the carriers with abnormal allele were pre-clinical HD patients or would be HD patients. III_{10}, III_{12}, III_{14}, IV_3, and V_2 had the same $(CAG)_{48}$; IV_{11} and IV_{12} carried $(CAG)_{67}$; and IV_{14} carried $(CAG)_{63}$ (Tab. 1). The number of CAG triplet repeats of normal persons in control group ranged from 8 to 25.

Tab.1 The relationship of disease conditions and (CAG) n

Members	Gender	Age (years)	Pathogenic $(CAG)_n$	Age of onset (years)
III_{10}	Male	49	48	33
III_{12}	Male	46	48	33
III_{14}	Male	43	48	33
IV_3	Male	35	48	unaffected
IV_{11}	Female	29	67	25 (possible)
IV_{12}	Female	24	67	17
IV_{14}	Female	22	63	21 (possible)
V_2	Female	7	48	unaffected

4 Discussion

The onset age of HD ranges from 35 to 50, and the patients tend to die 15 or 20 years later after the first onset [5]. There is no effective treatment for this hereditary neurodegenerative disease so far. Thus pre-clinical test and prenatal diagnosis are important for the individuals at risk of HD, for example, the people with HD family history. In this pedigree that we investigated, there are 15 members at high risk of HD, including two subjects with the possible symptom of early HD. Considering the pregnant person among the individuals at risk in this HD family, we carried out the genetic diagnosis in order to provide them with genetic counseling, and also to make preparation for the further study of the pathogenesis and treatment on HD.

With the discovery of pathogenic gene of HD by Huntington's Disease Collaborative Research Group, researchers realize that the longer CAG trinucleotide repeats serve as the etiological basis of HD[6]. PCR and DNA sequencing have been being employed to diagnose HD definitely. However, the resulting region containing the abnormal gene is a GC-rich region, which is difficult to amplify with routine PCR methods. We performed a modified touchdown PCR procedure to improve the specificity of the reaction and employed 5% of total PCR system DMSO to facilitate the denaturation of genomic DNA template. According to this protocol, we obtained the resulting PCR products with two primers in one reaction, instead of conducting nest-PCR with four primers in two reactions. Obviously, our method demands less time and less cost, which is of benefit to introducing such genetic diagnosis into clinic with less expense of patients. At the same time, the modified PCR protocol can be universally employed in genetic diagnosis of hereditary disorders caused by longer trinucleotide repeats.

According to the researches on different populations, the number of CAG trinucleotide repeats in normal individuals ranges from 6 to 35, but in HD patients ranges from 36 to 250[7]. In this Wuhan HD pedigree, genetic diagnosis showed that patients III_{10}, III_{12}, III_{14} and the abnormal allele carriers IV_3 and V_2 carried the same pathogenic gene with $(CAG)_{48}$, IV_{11} and IV_{12} carried the same pathogenic gene with $(CAG)_{67}$, and IV_{14} carried the abnormal allele with $(CAG)_{63}$. The number of CAG repeats in the control group ranged from 8 to 25.

HD is characterized by dynamic mutation of *IT15* during the abnormal gene transmitted from parents to the descendants[8,9]. Especially, the paternal transmission tends to cause the length of CAG repeats increasing[10,11], which in turn results in the genetic anticipation[12,13], that is, the offspring appear HD symptom at earlier age and with more severe conditions. There is obvious dynamic mutation in this family during the paternal *IT15* transmitted from III_8 to IV_{12}. All the five male patients of the third generation developed HD at 33 years old. Therefore, we speculated that the late III_8 should have the same number of CAG repeats, i.e. $(CAG)_{48}$, as III_{10}, III_{12}, and III_{14} carried. The late daughter of patient III_8 developed HD at 11 and died at 15; and his older daughter, the patient IV_{12} currently at age 24, has suffered HD for seven years. In addition, IV_{11} showed unstable gait

at her age of 23 and gradually developed to limp. The 22-year-old IV_{14} developed abnormal eyes movement half a year before, which is highly suspected as an early HD symptom[14]. Among the members at risk in the fourth generation, the CAG trinucleotide repeats expansion resulted from paternal transmission is responsible for the genetic anticipation.

According to the research on age of onset in different populations, it is widely believed that the age of onset is reversely related to the number of CAG trinucleotide repeats, that is, more CAG repeats implies earlier onset[15]. In this Wuhan family, the members in the fourth generation, with more CAG repeats than their fathers, tended to display HD much earlier than the patients in the third generation. However, previous studies on the monozygotic twins indicated that different living conditions and individual habits may result in distinct age of onset[16,17]. Some researchers have been striving to affirm some gene loci closely associated with the age of onset. In this pedigree, we found that IV_{11} and IV_{12} carried the same $(CAG)_{67}$, but they had different age of onset, 23 and 17, respectively. The number of CAG repeats in IV_3 was the same as the patients in the third generation, but at his 35 years, the abnormal allele carrier had not shown any HD symptom. We consider that gender, living conditions and some genetic loci may be responsible for the distinct age of onset in the carriers containing the same CAG repeats.

As for the associated pathogenesis, most researchers believe that HD possibly results from the formation of Htt aggregates and inclusions. Others hold the competitive opinions that polyglutamine fragment cleaved by enzyme should be easier to form aggregates than complete Htt. Besides, the energy metabolism disorder caused by excitotoxicity and oxidative stress may also engage in the pathogenesis of HD[18]. Since the uncertain pathogenesis, only some symptomatic treatments are used clinically at present, though various experimental therapies[19], for example, administration of Htt inhibitors, caspase inhibitors or neurophic factors, are in progress. Anyway, the preclinical diagnosis and prenatal genetic diagnosis are very helpful to control and prevent HD, especially for people with HD family history.

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武汉地区 CAG 扩增突变致青少年发病的亨廷顿舞蹈病的家系分析

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摘要：目的 对青少年发病的亨廷顿舞蹈病(Huntington disease)家系进行致病 *IT15* 基因早期诊断分析，为家系成员提供遗传咨询, 并为后续的HD发病机制及实验治疗研究提供依据。**方法** 按照知情同意原则抽取家系成员外周血，提取基因组 DNA，采用改良的降落 PCR 方法扩增 *IT15* 基因致病区域，DNA 测序检测异常等位基因(CAG) n 三核苷酸重复次数。**结果** 在该家系三代 25 名成员中，共发现 8 名致病 *IT15* 基因携带者，其中，III_{10}、III_{12}、III_{14}、IV_3 和 V_2 CAG 三核苷酸的拷贝数均为 48，IV_{11} 和 IV_{12} 均为$(CAG)_{67}$，IV_{14} 为$(CAG)_{63}$，而对照组 35 名正常人的CAG三核苷酸的拷贝数为 8-25，两者之间没有重叠。**结论** 家系中第四代致病基因携带者 IV_{14} 与第三代患者 III_{10} 比较，CAG 三核苷酸重复次数增加 15 次，即本家系 *IT15* 基因在传递过程中发生了扩增突变。同时，扩增突变导致该家系出现青少年发病及遗传早现现象。

关键词： 亨廷顿舞蹈病；早期诊断；扩增突变；遗传早现