Molecular genetics of Huntington’s Disease: When size does matter

Nagehan Ersoy
Halıç University, Department of Molecular Biology and Genetics, 34280, Istanbul, Turkey
(nagehanersoy@halic.edu.tr)

Received: 05 March 2007; Accepted 05 May 2007

Abstract

Huntington’s Disease (HD) is a late-onset and progressive neurodegenerative disease of the central nervous system with autosomal dominant inheritance. The prevalence of the disease is about 1/100 000 among individuals of European descent. The clinical symptoms of HD involve motor dysfunction, behavioural disturbances and cognitive decline. The pathology of HD is restricted to the brain, and the predominant neuropathological hallmark is selective loss of neurons within the striatum. The human HD gene (IT-15) was localized to chromosome 4p16.3 and consists of 67 exons spanning 180 kb of DNA. The mutation underlying disease is the expansion of the highly polymorphic CAG repeat tract in the first exon of the HD gene. In fact, HD belongs to a group of disorders for which the causative mutation is the expansion of CAG repeats in the respective genes. A total of nine such conditions have been described so far, which are collectively described as “Polyglutamine Diseases”. In HD, normal chromosomes possess 6-35 CAG repeats; mutated chromosomes carry 36-250 repeat units. There is a strong inverse correlation between the age of onset and expanded CAG repeat length. The direct molecular diagnosis for the disease is available since 1993, however genetic counseling protocols and ethical rules should be followed in order to avoid possible negative implications of a molecular test result.

Key Words: Huntington’s Disease, polyglutamine, neurodegeneration, CAG repeats

Huntington Hastalığı’nın moleküler genetiği

Özet


Anahtar Sözcükler: Huntington Hastalığı, poliglutamin, nörodejenerasyon, CAG tekrarları
Historical background of Huntington’s Disease

The first definitive description of Huntington’s Disease (HD) was given in 1872 by Dr. George Huntington in his article titled “On Chorea” (Huntington, 1972). This article pinpoints the cardinal features of the disease; its hereditary nature, manifestation in adult life, progressive nature and tendency of patients to insanity. Although this definition of the condition was not the first one, it was the first full outstanding description, which draws attention to the still-accepted marked features of the disease. Dr. Huntington called the disease “Hereditary Chorea”, however, later observations of some cases which do not present with chorea led to the adoption of the designation “Huntington’s Disease”. Besides summarizing the essential properties, this first full description has also directed the HD research to enlighten the origin and prevalence of the disease. It has become clear that the gene has a Northern European origin, with a prevalence of 5-10 affected people per 100 000 among individuals of European descent (Harper, 1992).

Clinical correlates

Huntington’s Disease is one of the chronic neurodegenerative disorders with autosomal dominant inheritance affecting the central nervous system (Gusella, 1995). Huntington’s Disease is considered as a late-onset disease since the first symptoms appear in the third to fifth decade of life. However, epidemiological studies have revealed a normal distribution of age of onset peaking at the fourth decade, with small numbers of patients manifesting the disease before 20 (5-10 % of HD cases) or after 60 years of age (~20 % of HD cases) (Harper, 1996). Juvenile cases, who present the disease before the age of 20, experience more severe and rapid disease course. The duration of illness is usually 15-20 years after disease onset (Craufurd, 1996; Bates et al., 1998).

The clinical symptoms of HD involve three basic abnormalities: Motor dysfunction, behavioural disturbances and cognitive decline (Craufurd, 1994). Motor abnormalities usually start with chorea, which involves involuntary and low-amplitude movements in the distal extremities, and progresses into continuous jerky movements, affecting all parts of the body. The severity of chorea progressively increases during the initial years of the illness, and is later replaced by bradykinesia and rigidity (Craufurd, 1996). In the disease course, patients also develop dysarthria, dysphagia, balance problems, and incoordination. As the disease progresses, rigidity dominates, leaving the patient bed-bound or confined to a wheelchair. In juvenile HD patients, rigidity is more common than chorea. Behavioral abnormalities in the form of personality changes, anxiety, irritability and depression usually precede the motor symptoms, but often neglected or not regarded as a disease sign (James et al., 1994). Huntington’s Disease patients show overall impairment in recognizing facial expressions. Suicide is more common in HD patients than in the general population, and is the third most common cause of death (Craufurd, 1994; Farrer et al., 1986). In the very late stages of the disease, mental activities become slower and patients develop dementia, characterized with poor concentration, inefficient use of memory, and impairment of executive functions (Craufurd, 1996).

Neuropathology of Huntington’s Disease

Morphological studies

The pathology of HD is restricted to the brain, and the predominant neuropathological hallmark is selective loss of neurons within the striatum. There is loss of medium sized spiny GABA-ergic striatal output neurons (up to 80 %), without significant loss of interneurons (Graveland et al., 1985). The pathological changes may be minimal in the early stages, however, in more advanced cases there may be widespread atrophy in the basal ganglia and the deep layers of the cortex. Post-mortem studies of HD brains revealed that neuronal loss probably begins in early life. At the time of onset of motor signs cortical grey matter, subcortical white matter, and about 30 % of caudate neurons are already lost (Figure 1). These all account for the loss of brain weight by 20 to 30 per cent less than normal (Monte et al., 1988). The neuronal intranuclear inclusions (NII) found in the brain are accepted as a neuropathological marker of HD. Huntingtonin inclusions were shown in the cortex and striatum of transgenic HD mice, and post-mortem HD brains (Davies et al., 1997; DiFiglia et al., 1997). The inclusions contain an N-terminal fragment of the
mutant protein, huntingtin (htt), and ubiquitin, which form prior to the development of a neurological phenotype.

**Neurotransmitter studies**

The neurotransmitters gamma amino butyric acid (GABA), acetylcholine and dopamine were found to be decreased in HD basal ganglia (Bier et al., 1997). Glutamic acid decarboxylase (GAD) levels, the enzyme involved in GABA biosynthesis, was also found to be reduced in the caudate, putamen and globus pallidus (Graybi et al., 1990). GABA is one of the neurotransmitters found in spiny neurons, so the finding of significantly reduced levels is consistent with the morphological observations.

**Molecular genetics of Huntington’s Disease**

**Huntington’s Disease gene structure**

The human HD gene was localized to chromosome 4p16.3 by positional cloning in 1993, after ten years of intense research (Huntington’s Disease Collaborative Research Group, 1993). The HD gene, called IT-15, consists of 67 exons spanning 180 kb of DNA(Figure 2). In exon 1, 17 codons downstream the ATG start codon, there is a polymorphic and unstable (CAG)$_n$ repeat tract coding for glutamines. The repeats vary in number from 6-35 in normal individuals and 36-250 in HD patients. Adjacent to (CAG)$_n$ repeats, there is slightly polymorphic CCG repeats (6-12 repeats), which are coding for prolines and are stably transmitted (Lin et al., 1995). Genotype-phenotype correlation studies revealed that most normal chromosomes and the majority of (>90 %) disease chromosomes possess seven CCG repeats (Andrew et al., 1994). In exon 58, there is a rare codon-loss polymorphism ($\Delta$2642, GAG), which occurs in five per cent of normal and 24-38 % of HD chromosomes. The codon loss itself does not seem to be deleterious, since normal homozygotes for this deletion have been reported (MacDonald et al., 1992). The human HD gene is highly conserved across species, including intron-exon boundaries. Mouse (Barnes et al., 1994), rat (Schmitt et al., 1995), fugu (Baxendale et al., 1995), zebrafish (Karlovich et al., 1998), and pig (Matsuyama et al., 2000) show very strong (80 %- 95 %) overall homology. Drosophila homologue is conserved less (Li et al., 1999), and HD gene has been reported to be absent in Caenorhabditis elegans and Saccharomyces cerevisiae (Strong et al., 1993).

**Figure 1:** Striatal sections from fixed cerebral hemisphere of an Huntington’s Disease patient (b) and age-matched control (a). There is loss of white matter in caudate nucleus, putamen and cortex (Vt: lateral ventricule, cd: caudate nucleus, put: putamen) (Bates et al., 2002).

**Figure 2:** Polymorphic repeats and codon-loss polymorphism in the IT-15 gene.

**Huntington’s Disease mutation**

The mutation underlying Huntington’s Disease is the expansion of the highly polymorphic CAG repeat tract in the first exon of the HD gene (Huntington’s Disease Collaborative Research Group, 1993). In fact, HD belongs to a group of disorders for which the causative mutation is the expansion of CAG repeats in the respective genes. A total of nine such conditions have been described so far, which are collectively described as “Polyglutamine (polyQ) Diseases”. These include Huntington’s Disease, Dentato-Rubral Pallidoluysian Atrophy, Spinal and Bulbar Muscular Atrophy and
Spinocerebellar Ataxia Types 1, 2, 3, 6, and 17 (Cummings and Zoghbi, 2000). Normal chromosomes possess 6-35 CAG repeats inherited in a Mendelian fashion; HD chromosomes bear 36-250 repeat units that are inherited in a non-Mendelian manner, because of repeat instability upon transmission. Moreover, rare alleles with 36-39 repeats were found in the unaffected elderly relatives of sporadic HD cases (Rubinsztein et al., 1996). Derived from the results of more than 1000 tests and reported population analyses, United States Huntington’s Disease Study Group has defined four CAG repeat size intervals associated with varying disease risk in HD (Figure 3).

Normal range: Alleles with 26 and less CAG repeats are not related to disease in any case. In addition, these alleles have never been expanded and cause disease in the next generation.

Meiotic instability range: Individuals with 27-35 CAG repeats in their IT-15 gene do not develop HD, but these alleles may show meiotic instability, increase in size and cause HD in the next generation (Telenius et al., 1995). For example, 27 CAG repeats in a healthy father has been reported expand to 38 repeats in his diseased son (McGlennan et al., 1995).

Reduced penetrance range (intermediate range): Only part of the individuals with 36-39 CAG repeats develop HD, in other words, the repeats in this range do not show full penetrance (Rubinsztein, 1996). Moreover, as the repeat numbers increase in this range, penetrance also increases. This kind of reduced penetrance is an extreme case, which implies that genetic background and environmental factors possibly play roles in the expression of the same mutation (Wells and Warren, 1998).

Pathological range: Individuals with 40 or more CAG repeats certainly develop the disease if they live long enough and there is 50% chance of inheriting the disease to the next generation.

**Repeat instability**

The CAG repeats in the HD gene are highly unstable, showing expansions and contractions in length over generations. In humans, the expanded CAG repeat tract is unstable on 80% of the transmissions. The size and direction of the instability may differ depending on the sex of the affected parent. In maternal transmissions, nearly equal numbers of expansions and contractions are observed, and shifts range from one to three repeats in size. In contrast, paternal transmissions are more frequently expansions, and in some cases increases in size can be ten or more. In addition to meiotic instability, modest degree of instability has been described within and between

---

**Figure 3.** CAG repeat sizes in the IT-15 gene and associated Huntington’s Disease risk (Wells et al., 1998)
somatic tissues of HD patients (Telenius et al., 1994). In the brain, instability was observed in the caudate and putamen; in non-CNS tissues, most instability was observed in the liver and kidney (Telenius et al., 1994). Three general mechanisms can be proposed to account for repeat instability: Slippage during DNA replication, misalignment with subsequent excision repair and unequal crossover and recombination. Slippage-mediated length change during DNA replication could better explain the increase in repeat size in HD (Richards and Sutherland, 1994).

Genotype-Phenotype relations

There is a strong inverse correlation between age of onset (AO) and expanded CAG repeat length (Stine et al., 1993; Brinkman et al., 1997). The most important factor affecting AO is the length of the expanded CAG repeat in the HD gene, accounting for about 70% of the variation in AO (Andrew et al., 1993; Hannan, 2004). However, since any expanded CAG repeat size can be associated with a broad range of onset ages, the number of repeats alone cannot be used to predict the AO (Snell et al., 1993; Rubinsztein et al., 1996). The second modifier of AO is the sex-of-parent effect, which accounts for 2-5% of the variation in AO (Farrer et al., 1992). Juvenile HD patients more likely inherit the disease from their fathers (Farrer et al., 1992) and patients with late AO more frequently inherit the disease from their affected mothers (Hall et al., 1983; Rubinsztein et al., 1996). The remaining variation in AO may partly be explained by environmental effects. However, recent studies suggest a strong genetic component (Djousse et al., 2003), which implies that there are other genes that modify AO in HD.

Reports of some HD patients with 36 repeats, and some very old individuals with 36-39 repeats but no recognizable HD symptoms proves that the disease is not always fully penetrant (Rubinsztein et al., 1996). Penetrance is defined as the proportion of individuals with a specified genotype who presents the expected phenotype in expected life span. Penetrance in HD increases with increasing repeat length in the intermediate range (36-39 CAG repeats) and there is complete penetrance with a CAG repeat size of greater than or equal to 40.

A distinctive feature of HD is genetic anticipation, meaning that, symptoms appear at earlier ages with greater severity in successive generations. It is now clear that anticipation is due to the instability of the expanded repeat sequence over generations. As it passes through the germline, particularly the paternal line, the number of repeats tend to increase, resulting in earlier AO and more severe disease course (Jennings, 1995).

New mutations, resulting in de novo disease presentation have been described in HD (Goldberg et al., 1993). Higher normal repeats may expand into the pathological range and may cause disease in the next generation, leading to a new mutation in the family. The criteria for the identification of a new mutation is that the parents of the sporadic case must have lived beyond the expected age of onset without any manifestations of the disease, and paternity of the sporadic case must be confirmed (Harper, 1992).

Molecular diagnosis of Huntington’s Disease and ethical issues

Before the identification of the HD mutation, molecular diagnosis was only possible via linkage analysis. Identification of the mutation in 1993 and rapid technical advances have made the molecular diagnosis of HD easier, more reliable and faster. Molecular diagnoses of HD and other polyQ diseases are relatively simple since there is only one mutation underlying the disease condition. In direct mutation analysis, the CAG repeat region in the IT-15 gene is amplified with PCR, subjected to electrophoresis and the repeat numbers are calculated after Southern blotting or via fluorescent detection of the PCR products (The American College of Medical Genetics / American Society of Human Genetics Huntington Disease Genetic Testing Working Group, 1998). However, where the disease is a late-onset, progressive, autosomal dominant and incurable condition, precautions should be taken regarding the possible negative outcomes of a molecular test result. Therefore, genetic counseling protocols and ethical rules have been developed to complement the direct mutation analysis procedure for HD. Huntington’s Disease was the first late-onset disease for which direct molecular diagnostic testing became possible, thus counseling issues were first developed for this condition, but later accepted with slight modifications for similar diseases with autosomal dominant inheritance. The implications of a favorable or
unfavorable test result on patient’s and relative’s life should be widely discussed in serial meetings before starting the test procedure and even before drawing the blood sample. It should be emphasized that the test result will obviously effect the presymptomatic and at-risk people in the family (International Huntington Association and the World Federation of Neurology Research Group on Huntington’s Chorea, 1994). The molecular test should not be offered to people who have not taken adequate genetic counseling or to those who are not psychologically competent to infer healthy judgements about the result. This issue becomes especially important for presymptomatic diagnosis. Moreover, it should be the patient’s own decision to take the molecular test, any pressure from family members or medical team should be avoided. According to the internationally accepted rules, it is forbidden to share the test results with third parties without the written permission of the patient. It is also not ethical to disclose the result on the phone or by mail (Craufurd, 1996).

In addition, there is still considerable concern that genetic information will be used unfairly in providing life insurance or employment. In this situation, the desire of the patients to avoid the transmission of the disease to their children may conflict with the possible adverse effects of diagnosis in the at-risk patient. This dilemma had lead to the development elaborate protocols to ensure that at-risk individuals understand and become emotionally competent to accept all of the implications of diagnosis.

References


Bates GP, Mangiarini L, Wanker EE and Davies SW. Polyglutamine expansion and Huntington’s disease.


Stine OC, Pleasant N, Franz ML, Abbott MH, Folstein SE,


