A systematic review of the intergenerational aspects and the diverse genetic profiles of Huntington's disease

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ABSTRACT. Huntington's disease (HD) is a rare progressive and fatal neurogenetic degenerative disease, characterized by movement and personality disorders and by progressive dementia. Its prevalence varies by ethnic origin and different genetic profiles predisposing individuals to HD in each population. The prevalence of HD is 5-10 per 100,000 individuals in Caucasian populations of North America and Western Europe. It is an autosomal dominant disease associated with the expansion of CAG-type repetitive DNA sequences in the *HTT* gene. This gene, located on the short arm of chromosome 4, encodes the protein huntingtin. In this study, we reviewed 17 articles about HD that report data from 2400 affected individuals from various countries around the world, including Venezuela, China, Croatia, Turkey, Germany, Italy, Brazil, Spain, Taiwan, India, the Netherlands, Russia, and the USA, with a focus on genetic profiles and intergenerational expansions or contractions of expanded alleles responsible for causing HD. We discuss the genetic characteristics of HD in different

populations and any atypical cases reported in these studies.

Key words: Huntington's disease; CAG repeats; Intergenerational aspects

INTRODUCTION

Huntington's disease (HD) is a rare progressive and fatal neurodegenerative disease, characterized by a lack of motor coordination of voluntary and involuntary muscles, known as choreic movement, personality disorders, and progressive dementia (Chandler et al., 1960). The genetic profiles of HD and the intergenerational aspects of expanded allele transmission vary across different regions of the world. The prevalence of HD varies with ethnic origin, with Caucasian populations of North America and Western Europe having 5-10 subjects affected by HD per 100,000 people (Lima et al., 2000; Hormozian et al., 2004; Gil and Rego, 2008). Together with literature reports of atypical cases of HD, the above-mentioned variations indicate that there is a need for a systematic review of the HD literature. To this end, this review aggregates information about the genetic profiles of HD in different populations and atypical cases.

HD is caused by a mutation triggered by variations in the number of CAG repeats in unstable DNA regions of the *HTT* gene, which is located on the short arm of chromosome 4 (4p16.3). The mutable region is located in the first exon of *HTT* and encodes a polyglutamine N-terminal tail of the encoded protein, huntingtin (Wexler et al., 2004). Alleles that bear 27 or fewer copies of the CAG repeat result in a normal phenotype. Intermediary alleles, which have 27-35 CAG repeats, are unstable and can be transmitted as an expanded allele to offspring; nevertheless, carriers of the intermediary allele type also have a normal phenotype. However, intermediary alleles get expanded mainly during male gametogenesis, which undergoes a greater number of cycles of division and duplication of genetic material than oogenesis. Hence, there is a greater probability of an error occurring in DNA replication during spermatogenesis, which increases the risk of mutated alleles being inherited from the father (Wheeler et al., 2007).

Alleles with 36-39 CAG units have reduced penetrance and generate both a normal phenotype as well as some rare cases of HD. Alleles with >39 CAG copies show complete penetrance and inevitably cause, at some stage of life, the HD phenotype.

Classical studies examining the geographical distribution of HD, along with several epidemiological studies, suggest that the alleles responsible for causing HD originated from a single mutation in a common ancestor from Western Europe, and this mutation then spread to other regions of the world as a result of migration. Different haplotypes that include the CCG repeat region, which is adjacent to the CAG region, were identified in different populations and confirm the existence of mutations that had independent and distinct origins from that of a single common HD ancestor (García-Planells et al., 2005).

In patients with HD, the onset of clinical symptoms usually starts between the ages of 35-55, although the disease can manifest after the age of 80, or much earlier, in teenagers or children, because of the phenomenon of anticipation, which occurs in 20% of cases of HD. Approximately 10% of patients with HD have onset of clinical manifestations before the age of 20, and 5% before the age of 14. If the expanded allele transmitted to the offspring is of maternal origin, an expansion of >20 units is very rare (Andrew et al., 1993; Nahhas et al., 2005).

The incidence of genetically confirmed cases of HD in individuals who have no family history of HD and who would therefore be the first confirmed case in the family is greater than 8%. These cases are caused by new, sporadic expansions of intermediary alleles. Such unstable alleles favor the emergence of mutated alleles in offspring, which leads to HD (Nahhas et al., 2005).

From the onset of initial symptoms, HD will progress to death within 15-20 years (Ho et al., 2001). Variability in clinical signs and symptoms of the disease often requires a precise molecular investigation at the genetic level to enable final diagnosis (Stevanin et al., 2003).

HD is an autosomal dominant genetic disease. Each child of an affected parent has a 50% chance of developing the disease; therefore, only one allele inherited from the mother, or from the father, is able to cause the disease in the offspring. However, although very rare, when in the homozygous state, the 2 expanded alleles result in increased physical deterioration of HD-affected individuals when compared to affected individuals bearing only one expanded allele. Studies further suggest that homozygosity in the expanded alleles does not lead to an earlier onset of the disease, but changes the severity of HD symptoms and their progression (Squitieri et al., 1994).

MATERIAL AND METHODS

A search was conducted on the Internet using databases related to the health sciences, such as Pubmed/Medline, Scielo, and Bireme. Articles were selected from these 3 databases. The key words used were "Huntington's disease" and "CAG repeats". Advanced search functions were used to restrict the search to articles published from 2000-2012.

RESULTS

We identified 616 scientific articles published from 2000-2012, and 41 of them were selected according to the chosen theme. Of the 41 articles, 17 were chosen because they reported detailed molecular investigations of HD, intergenerational analysis of expanded alleles in different regions of the world, and atypical cases of the disease (Table 1). The 2381 individuals investigated who were described in these papers are the subject of discussion in this systematic review. Of the selected articles, 8 reported anticipation in HD-affected patients, and 5 articles discussed cases of juvenile HD primarily caused by paternal inheritance.

Studies conducted in Venezuela on 112 individuals with HD confirmed the high instability of alleles transmitted through the father, as indicated by the higher frequency of juvenile HD with paternal inheritance (Wheeler et al., 2007). However, 2 studies of juvenile HD detected no significant difference in the frequency of HD between the paternal and maternal routes of inheritance (Nahhas et al., 2005).

In 2005, a molecular investigation was conducted on 2 individuals who had inherited HD with anticipation from the mother. She carried an allele with 70 CAG repeats, and had transmitted an allele containing 130 CAG copies (Nahhas et al., 2005).

Few articles have reported data on Brazilian HD patients. These articles reported investigations of CAG repeats-containing alleles at the molecular level (Lima et al., 2000; Raskin et al., 2000; Agostinho et al., 2012). One of these papers correlated genetic character-

Table 1. Selected articles of this review about diverse genetic profiles and intergenerational transmission of Huntington's disease (HD) in different regions of word.

Authors and years	Experimental mode	Study design	Number of individuals investigated	Methods	Objective	Place of study	Results
(Andresen et al., 2007)	HD-affected children	Experimental	443	Molecular analysis of polymorphic CAG region	Relate polymorphic CAG and CCG regions to age at onset of HD	Venezuela	Size of CAG repeats explain the variation in HD age of onset
(Wheeler et al., 2007)	Sperm and blood samples of individuals affected	Experimental	112	Molecular analysis of polymorphic CAG region	Investigate factors associated with intergenerational instability in paternal transmission	Venezuela	Intergenerational instability in paternal transmission
(Akbas and Erginel-Unaltuna, 2003)	Adult individuals of both genders	Experimental (case control study)	127	Molecular analysis of polymorphic CAG region	Investigate CAG repeats of the *HTT* gene	Turkey	Juvenile-onset HD occurs more frequently in paternal transmissions
(Hećimović et al., 2002)	HD-affected individuals	Experimental (case control study)	44	Molecular analysis of 3 polymorphic regions related to HD	Investigate CAG and CCG repeats of the *HTT* gene	Croatia	Genetic origin of the *HD* gene in Croatia comes from Western Europe
(Tang et al., 2006)	One family with HD history	Experimental (case control study)	15	Molecular analysis of polymorphic CAG region of HD family	Investigate the intergenerational transmission of the *HD* gene	China	Expanded allele is more frequent in paternal transmission
(Metzger et al., 2006)	HD-affected individuals	Experimental	980	Molecular analysis of polymorphic CAG and CCG regions	Relate polymorphic CAG and CCG regions to age at onset of HD	Germany, Italy, and other countries of Europe	Size of CAG and CCG repeats explain the variation in HD age of onset
(Wang et al., 2004)	HD-affected individuals	Experimental (case control study)	53	Molecular analysis of polymorphic CAG and CCG regions	Relate polymorphic CAG and CCG regions to age at onset of HD	Taiwan	Size of CAG repeats explain the variation in HD age of onset and CCG length is not related to HD onset of age
(Garcia-Planells et al., 2005)	HD-affected individuals	Experimental	115	Molecular analysis of 6 polymorphic regions related to HD	Investigate the origin of the *HD* gene in Spain	Spain	Size CAG repeats is related to HD age of onset and the origin of the *HD* gene of Spanish population comes from Western Europe
(Andrich et al., 2008)	One HD-affected individual	Experimental (case control study)	1	Molecular analysis of polymorphic CAG region	Relate HD clinical symptoms in an individual with HD intermediary allele	Germany	Supposes HD intermediary allele may cause HD phenotype
(Kartsaki et al., 2006)	Individuals with HD family history	Experimental	33	Molecular analysis of polymorphic CAG region	Intergenerational study of HD families	Crete	Genetic profile of Crete population is 36-42 CAG repeats, the onset-juvenile HD cases were caused by maternal and paternal transmission
(Pramanik et al., 2000)	HD-affected individuals	Experimental	28	Molecular analysis of polymorphic CAG and CCG regions	Investigate the origin of the *HD* gene and genetic profile of India population	India	CCG allele with four repeats was found in Indian population and genetic profile is 41-56 CAG repeats

Table 1. Continued.

Authors and years	Experimental mode	Study design	Number of individuals investigated	Methods	Objective	Place of study	Results
(Nahhas et al., 2005)	HD-affected individuals	Experimental (case control study)	2	Molecular analysis of polymorphic CAG region	Investigate maternal transmission of the *HD* gene	USA	Very large expansions can also occur through the maternal lineage
(Siesling et al., 2000)	HD-affected individuals	Experimental (case control study)	172	Molecular analysis of polymorphic CAG region	Investigate clinical diagnostic mistakes of HD	Netherlands	There are mistakes in clinical diagnosis of HD
(Kutuev et al., 2004)	HD-affected individuals	Experimental	59	Molecular analysis of polymorphic CAG and CCG regions	Intergenerational study of HD families	Russia	High instability of the *HD* gene in paternal transmission
(Raskin et al., 2000)	HD-affected individuals	Experimental (case control study)	92	Molecular analysis of polymorphic CAG region	Investigate CAG repeats of the *HTT* gene	Brazil	Expanded allele is more frequent in paternal transmission and Brazilian genetic profile is 39-88 CAG repeats
(Lima et al., 2000)	HD-affected individuals	Experimental (case control study)	44	Molecular analysis of polymorphic CAG region	Relate polymorphic CAG regions to age at onset of HD	Brazil	Brazilian genetic profile is 43-53 CAG repeats and CAG size variation has no difference between paternal and maternal transmission
(Agostinho et al., 2012)	HD-affected individuals	Experimental	61	Molecular analysis of polymorphic CAG and CCG regions	Investigate CAG and CCG repeats of the *HTT* gene	Brazil	CAG expansion varied from 12 to 58 repeats in the 122 alleles tested and 86.9% of these alleles had 7 CCG repeats

istics of HD with the age at onset of the clinical symptoms (Lima et al., 2000). A case-control study investigated 84 HD-affected individuals. In another study, the shortest HD allele identified contained 39 CAG repeats. An inverse correlation between the onset of clinical manifestations and the size of the CAG-repeat regions was also observed (Raskin et al., 2000). Furthermore, in subjects showing trinucleotide expansions, these expansions were longer when the mutated allele was inherited from the father than when it was inherited from the mother (Raskin et al., 2000).

In 2012, a molecular study in Brazil reported that the length of the CAG expansion varied from 12-58 repeats in 122 alleles tested. In the heterozygous subjects (N = 46), who carried at least one expanded allele each, the normal alleles contained 12-26 CAG repeats with a mean of 16 ± 3.3 and a median of 15. The shortest expanded allele that caused an HD phenotype contained 37 CAG repeats, and the longest contained 58 repeats. None of subjects was homozygous for an expanded CAG allele (Agostinho et al., 2012).

In the same study, repeats of 7 CCG trinucleotides were found in 78.7% of the sample. The CCG alleles, linked to the expanded CAG alleles had 6, 7, 8, or 10 CCG repeats (mean 7.1 ± 0.7), and 86.9% of these alleles had 7 CCG repeats. This was the first investigation of the polymorphic CCG region in Brazil (Agostinho et al., 2012).

In Turkey, a study of 127 individuals showed that the shortest HD-causing allele contained 38 CAG copies, and the longest allele contained 78 CAG repeats (Akbas and Erginel-Unaltuna, 2003).

Another survey conducted on the Crete island, Greece, studying HD in 33 individuals, reported that the shortest HD-causing allele contained 36 CAG copies and the longest allele had 42 CAG repeats (Kartsaki et al., 2006). In India, the shortest HD allele identified contained 41 CAG repeats and the longest allele, 56 CAG repeats (Pramanik et al., 2000).

Furthermore, it was in India where, for the first time, 4 CCG repeats were found adjacent to the CAG region in a subject with HD. The CCG region next to the CAG region has also been the target of many molecular studies. These studies are useful for confirming the results of molecular diagnosis or for the genetic study of the ancestral origin of causative HD alleles. Most of the articles that have analyzed the CCG regions reported that the genetic origin of the expanded HD alleles was Western Europe (Hećimović et al., 2002; García-Planells et al., 2005).

An experimental study with 53 patients suggested that the size of the CCG repeats is not a determining factor for the age of onset of HD (Wang et al., 2004), a finding that was confirmed by Agostinho et al. (2012). On the other hand, a study with 980 individuals in European countries provided strong evidence for an association of the size of the CAG and CCG repeats with age at onset of HD (Metzger et al., 2006).

Among the individuals studied in Brazil, the smallest allele causing onset of HD contained 37 CAG repeats and the longest allele had 88 CAG repeats (Lima et al., 2000; Raskin et al., 2000; Agostinho et al., 2012). In 2008, an atypical case was reported from Germany of a patient who was affected by HD even though the disease allele contained only 34 copies of the CAG repeat (Andrich et al., 2008).

DISCUSSION

HD is a disease of adults. Juvenile cases are rare (only approximately 10% of total cases), and the majority of the affected individuals inherit expanded alleles from their fathers

(Nahhas et al., 2005). However, one study showed no significant difference in the number of cases of juvenile HD between paternally or maternally inherited HD (Lima et al., 2000). The CAG repeat in a maternal allele usually does not expand to more than 20 copies (Nahhas et al., 2005; Kenney et al., 2007); however, a report in 2005 showed that an affected mother had transmitted a double-size allele to her daughter (Nahhas et al., 2005).

Two case reports in the literature have identified individuals who carried intermediary alleles and who were clinically diagnosed as being affected by HD. In one article, an individual who had an allele with 29 CAG repeats was diagnosed with HD by *post-mortem* autopsy (Andresen et al., 2007). Another article has reported that an individual who presented cortical atrophy, disorder of movement, and cognitive impairment carried an allele with only 34 copies of the CAG repeat (Andrich et al., 2008). However, neither study mentions any attempts by investigators to diagnose a Huntington-like disease.

Among the studies on HD, where differential diagnosis for other similar diseases was performed, the smallest number of CAG units associated with the HD phenotype was 37, identified in a patient of European descent. The absence of the clinical symptoms of HD has not yet been documented in any individual carrying an allele containing >39 CAG repeats.

Alleles with 7 CCG units were observed in 95% of cases. This finding might mean that those alleles had a genetic origin in Western Europe, the presumed location of a founder effect for HD. On the other hand, alleles containing 10 CCG units may have been the result of independent mutations that recently originated from other geographical regions. Usually these CCG alleles possess 7 or 10 repeats, with the exception of a case, reported by Pramanik et al. (2000), in which 4 CCG units were identified.

In conclusion, a molecular test that determines the length of a CAG repeat is essential because it can provide a definite diagnosis of HD. It is important to note that only about 40% of the carriers of the reduced penetrance allele may show the HD phenotype. Furthermore, during clinical investigation, the following procedures must be taken into account: differential diagnosis for atypical cases must be performed, along with paternity tests to confirm the origin of the disease alleles, mainly for new cases in the family.

In addition, it is difficult to define the smallest size of expanded CAG repeats responsible for the HD phenotype because there are many differences among individuals and populations. A definite clinical diagnosis often requires caution because the clinical presentation of signs and symptoms of HD is variable.

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