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Review

Intermediate alleles of Huntington's disease *HTT* gene in different populations worldwide: a systematic review

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ABSTRACT. Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder caused by a dynamic mutation due to the expansion of CAG repeats in the *HTT* gene (4p16.3). The considered normal alleles have less than 27 CAG repeats. Intermediate alleles (IAs) show 27 to 35 CAG repeats and expanded alleles have more than 35 repeats. The IAs apparently have shown a normal phenotype. However, there are some reported associations between individuals that bear an IA and clinical HD signs, such as behavioral disturbs. The association of IAs with the presence of clinical signs gives

clinical relevance to these patients. We emphasized the importance of determining the frequency of IA alleles in the general population as well as in HD families. Therefore, the aim of this study was to conduct a systematic review, in order to investigate the frequency of IAs in the overall chromosomes of different ethnic groups and of families with HD history worldwide as well as the frequency of individuals who bear the intermediate alleles. We searched indexed articles from the following electronic databases: U.S. National Library of Medicine and the National Institutes of Health (PubMed), Pubmed Central (PMC) and Virtual Health Library (VHL). Therefore, 488 articles were obtained and, of these, 33 had been published in more than one database. We accepted the article of only one database and ended up with 455 articles for this review. The frequency of IAs within the chromosomes of the general population ranged from 0.45 to 8.7% and of individuals with family history of HD ranged from 0.05 to 5.1%. The higher frequency of IAs in the general population (8.7%) was found in one Brazilian cohort.

Key words: Huntington's disease; Intermediate alleles; Prevalence; CAG repeats

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder, characterized by motor, cognitive, and behavioral impairment. HD is caused by a dynamic mutation due to the expansion of CAG repeats in the *HTT* gene (4p16.3). The *HTT* gene is responsible for coding a protein called huntingtin (HTT) whose main function is related to the transport of vesicles inside the cells (Gusella et al., 1983; Martin and Gusella, 1986; The Huntington's Disease Collaborative Research Group, 1993).

The prevalence of the disease varies with ethnicity. The prevalence for Caucasians in the Western Europe is estimated to be 5-10/100,000 individuals. The considered normal alleles have less than 27 CAG copies leading to normal phenotype. Intermediate alleles (IAs) show 27 to 35 CAG repeats and present a normal phenotype; however, these alleles are considered to be genetically unstable. Alleles with 36-39 CAG units show reduced penetrance, and may generate either a normal or an HD phenotype. Alleles with more than 39 CAG copies show full penetrance and determine, inevitably, the HD phenotype at some stage of life (The American College of Medical Genetics/American Society of Human Genetics Huntington Disease Genetic Testing Working Group, 1998). Due to the expansion of CAG repeats greater than normal threshold (up to 26 CAG repeats), the HTT gains an extra polyglutamine tail at the N-terminal region, and once expanded (>35 CAG repeats), HTT can be cleaved into fragments by proteases such as caspases and calpains. These protein fragments accumulate in specific regions for example, the medium spiny neurons inside nerve cells causing neuronal alteration (The Huntington's Disease Collaborative Research Group, 1993; Reilmann et al., 2014).

Many studies suggest the existence of factors that interfere with the stability of the CAG trinucleotide region across the generations. Some factors are the following: size of the CAG alleles, sex and age, as well as the presence of cis and trans factors and environmental ones (Pearson et al., 2005).

Some SNPs (single nucleotide polymorphisms) were reported as factors that could interfere in genetic stability of the CAG polymorphic region (*HTT*). It is believed that a specific SNP profile can determine the degree of predisposition in the event of CAG expansion at the *HTT* gene (Warby et al., 2009; Kay et al., 2015).

Different SNP haplogroups have been built in order to categorize individuals with normal CAG alleles and expanded ones in different ancestral origin populations (Warby et al., 2009). According to each genetic profile, these subjects are classified in haplogroups A, B, or C. In a sample of the European population, as known as of a high HD prevalence, it was observed that the haplogroup A is found in 95% of the affected individuals and, interestingly, the haplogroup A is also present in 53% of chromosomes of the general population (<27 CAG) (Warby et al., 2009). The haplogroups B and C have been found in populations that had low HD prevalence (Warby et al., 2009; Kay et al., 2015).

New HD mutations do not occur randomly on a chromosome. There is a greater predisposition of individuals with haplotype A to present HD (Squitieri et al., 1994; Warby et al., 2011).

The haplotype A, found in higher frequency in HD chromosomes of affected Caucasians (Western Europe), is also found in the chromosomes that bear IAs from the same ethnic group. Individuals with expanded alleles, as well as those with IAs, may have similar factors for predisposition and occurrence of CAG expansion (Goldberg et al., 1995; Warby et al., 2011).

The molecular mechanism that contributes to the occurrence of new HD mutations occurs step by step, expanding the polymorphic CAG region of a normal allele across the generations. This may be due to a genetic instability that causes multiple expansions to become IAs (27-35 repeats), reduced penetrance alleles (36-39 repeats), or complete penetrance alleles (>39 repeats) (Warby et al., 2009).

The IAs show genetic instability and the individuals who bear them can transmit an expanded allele to his/her offspring, giving rise to new cases of HD. The longer is the CAG expansion, the higher will be the chance of transmission of an expanded allele to the next generation (Nahhas et al., 2005; Wheeler et al., 2007; Warby et al., 2009). The risk of transmitting an expanded allele, with complete penetrance, to the offspring is higher when the IA shows more than 30 CAG repeats (Semaka et al., 2013a).

Interestingly, some authors have observed patients bearing IAs with behavioral changes (apathy and tendency to suicide), as well as some motor and cognitive changes. The association of IAs with the presence of clinical signs increases the clinical relevance to these patients. These results have important implications not only for the pathogenesis of the disease, but also for genetic counseling of HD, because in several cases, these individuals receive genetic counseling with the guarantee that they will be asymptomatic (Ha et al., 2012; Squitieri and Jankovic, 2012; Feigin, 2013).

Because of the risk of expansion and the consequent emergence of new mutations from IAs, we emphasize the importance of determining the frequency of IA alleles in the general population and within families with HD history. Therefore, the estimate of this frequency will be useful for the knowledge of the epidemiological profile of HD in Brazil. To our knowledge the prevalence of HD for the overall population in Brazil has not yet been estimated (Goldberg et al., 1993; Feigin, 2013; Semaka et al., 2013a; Agostinho et al., 2015).

The aim of this study was to conduct a systematic review to investigate the frequency of IAs on the overall chromosomes of different ethnic groups and of families with HD history worldwide as well as the frequency of individuals who bear the IAs.

MATERIAL AND METHODS

Search strategy

The systematic review was conducted searching indexed articles in the following electronic databases: U.S. National Library of Medicine and the National Institutes of Health (PubMed), Pubmed Central (PMC), and Virtual Health Library (VHL). The key words used were intermediate alleles and Huntington's disease.

It is important to mention that the filters differ from an electronic database to another. Due to the unavailability of the desired filter in one database, the inclusion criteria selected were those common to the other databases. The following filters were chosen: 1) full text and publication year (2000-2015) for searching in VHL; 2) full text, publication year (2000-2015) and human species for PubMed; and 3) for PMV, all kinds of available articles published and added in the last 15 years (2000-2015).

Inclusion/exclusion criteria

The articles chosen for this review were those which showed the number of IAs on the chromosomes of the general population and/or of people having family history of HD, as well as articles which dealt with the frequency of IAs in individuals of both groups. In order to avoid biases during categorization of IAs, only articles that reported individuals who bore 27 to 35 CAG repeats were included in this review. We exclude review articles, case reports, and articles with just free available abstract. If one article was published in more than one database we accepted only one.

RESULTS

According to the search strategy, 488 articles were obtained (33 from VHL, 422 from PMC, 28 from Pubmed, and 5 by manual search), and of these, 33 had been published in more than one database (25 in VHL and Pubmed, 3 in VHL and PMC, and 5 in PubMed and PMC). Finally, we ended up with 455 articles showed in the flowchart below (Figure 1).

The total numbers of subjects, reported in these articles were 3822 among HD-affected people and 4402 among unaffected ones. Many articles do not discriminate between the frequencies of individuals carrying IAs present in healthy families from those present in HD families. Therefore, it has been difficult to fully understand their results.

The frequency of IAs/total chromosomes reported in the articles, considering the general population, ranged from 0.45 to 8.7% (Raskin et al., 2000; Pulkes et al., 2014) and the frequencies of individuals who bore IAs and had family history of HD ranged from 0.05 to 5.1% (Raskin et al., 2000; Baine et al., 2013; Killoran et al., 2013; Pulkes et al., 2014).

Considering the genetic profile of *HTT* polymorphic CAG alleles, the shortest normal allele found in the general population, cited in this review, had seven CAG repeats and was found in Brazil (Raskin et al., 2000), on the other hand, the longest expanded allele had 122 CAG repeats and was found in Venezuela (Paradisi et al., 2008). It is important to mention that Nance et al. (1999) showed a CAG trinucleotide repeat expansion of approximately 250 repeats in a patient with juvenile HD - the largest CAG expansion reported within the huntingtin gene; however, our search took into consideration only articles from 2000 to 2015 (Nance et al., 1999).

It is worth mentioning that two methodologies have been adopted concerning the investigation of IAs. One considers the number of IAs/total number of chromosomes (allelic frequency - Table 1), and the other considers the number of individuals who bear an IA in a population (Table 2).

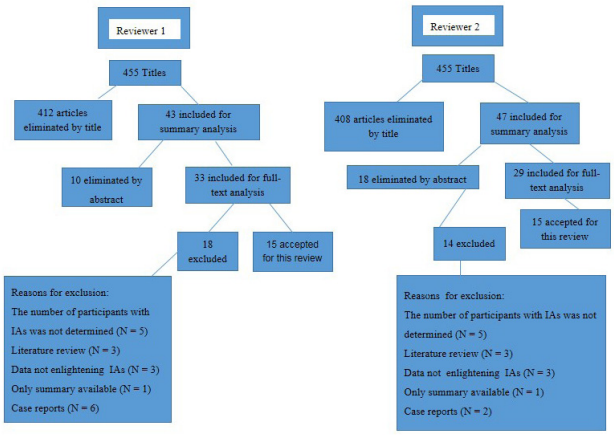

Figure 1. Flowchart showing the articles found by two independent researchers.

Table 1. Articles reporting the frequencies of intermediate alleles (IAs) per total of chromosomes, in the general population and within Huntington's disease (HD) families.

Title	Author/year	Methodology	Local	IA (%)	
				HD families	General population
CAG - Expansion haplotype analysis in a population with a low prevalence of HD	(Pulkes et al., 2014)	Sequencing analysis	Asia	-	0.45%
Large, normal, and reduced penetrance alleles in HD: instability in families and frequency at laboratory, clinic, and population	(Sequeiros et al., 2010)	Automated capillary electrophoresis	Latin America	3.5%	3%
HD DNA analysis in the Brazilian population	(Raskin et al., 2000)	Sequencing analysis	Latin America	-	8.7%
CAG repeat at the HD gene in the Portuguese population: insights into its dynamics and to the origin of the mutation	(Costa et al., 2006)	Polyacrylamide gel electrophoresis (6%)	Latin America	<2%	3%
Molecular diagnosis of HD in Brazilian patients	(Lima e Silva et al., 2000)	Polyacrylamide gel electrophoresis (6%)	Latin America	-	7%

CAG = cytosine, adenine, and guanine.

Table 2. Articles reporting the frequencies of subjects carrying intermediate alleles (IAs) in the general population and within Huntington's disease (HD) families.

Title	Author/year	Methodology	Local	IA	
				HD families	General opulation
Huntington CAG repeat size does not modify onset age in familial Parkinson's disease: The Gene PD Study	(McNicoll et al., 2008)	Polyacrylamide gel electrophoresis (6%)	Europe, North America and Oceania	-	5.2%
Intermediate CAG repeats in HD: Analysis of COHORT	(Ha et al., 2012)	CAG genotyping performed and detection of mtHTT and tHTT in a GLP HTRF assay	North America and Oceania	2.5%	-
Characterization of the Huntington intermediate CAG repeat expansion phenotype in PHAROS	(Killoran et al., 2013)	Polyacrylamide gel electrophoresis (5%)	ND	5.1%	-
Prenatal testing for HD in the Netherlands from 1998 to 2008	(van Rij et al., 2014)	CAG sizing not described	Europa	4%	-
Haplotype analysis of the CAG and CCG repeats in 21 Brazilian families with HD	(Agostinho et al., 2012)	Automated capillary electrophoresis	Latin America	4.1%	-
Clinical and genetic characteristics of Mexican HD patients	(Alonso et al., 2009)	Gel electrophoresis polyacrylamide.	Latin America	0.3%	
HD mutation in Venezuela: age of onset, haplotype analyses, and geographic aggregation	(Paradisi et al., 2008)	Polyacrylamide gel (8%) electrophoresis	Latin America	-	<0.05
Genetic features of HD in Cuban population: implications for phenotype, epidemiology, and predictive testing	(Vázquez-Mojena et al., 2013)	Polyacrylamide gel electrophoresis	Latin America	-	3.97%
HD in the South African population occurs on diverse and ethnically distinct genetic haplotypes	(Baine et al., 2013)	Gel electrophoresis on 6% polyacrylamide	Africa	<0.05	-
High frequency of IAs on HD-associated haplotypes in British Columbia's general population	(Semaka et al., 2013b)	Automated capillary electrophoresis	Europe	-	5.8%

CAG = cytosine, adenine, and guanine; mtHTT = mutant huntingtin; tHTT = total huntingtin; HTRF = homogeneous time resolved fluorescence; GLP = good laboratory practice; ND = data not available.

Regarding the geographic regions where the individuals were investigated, 52.94% were from Latin America, 11.76% from Europe, and 5.88% from either Asia or Africa.

Approximately 35% of the articles included in this review report the frequencies of subjects who bear the IAs in the general population, and approximately 47% report the frequencies of individuals bearing IAs with family history of HD. On the other hand, 17.64% of the studies did not discriminate between the two types of samples.

Of the nine studies conducted in Latin America, three of them were developed in Brazil. These studies show that there are differences in the number of CAG repeats in relation to the shortest and the longest alleles found: the three shortest normal alleles had 7, 12, or 16 repeats and the three longest expanded alleles had 58, 73, or 88 repeats (Lima e Silva et al., 2000; Raskin et al., 2000; Agostinho et al., 2012).

Lima e Silva et al. (2000) consider IAs within the range of 35 to 40 CAG, differently from what is considered by the American College of Medical Genetics. However, according to the running criteria, we calculated the frequency of individuals bearing IAs as 7% for that paper.

The frequencies of IA in the Brazilian general population ranged from 7 to 8.7% (Lima e Silva et al., 2000; Raskin et al., 2000). The highest frequency (8.7%) was found in 92 control participants composed by 50 Caucasians and 42 African-Brazilians. The authors suggest that 1/12 individuals in the general Caucasoid Brazilian population and 1/10 individuals in the African-Brazilian general population carry an IA. In this study, the CAG repeats in the Caucasoid sample ranged from 7 to 33 repetitions (average of 17.7 CAG repeats) and from 13 to 30 CAG repeats (mean 17.9 CAG) in the African-Brazilian sample (Raskin et al., 2000).

It is clear that the prevalence of IAs varies within populations of different ethnic origins. In a study conducted in Western Europe, there was a high frequency of people carrying IAs in the general population (5.8%) considering 1594 participants, i.e., 1/17 people is carrying an IA; among them, 2.3% had 27-30 CAG repeats and 0.6% had 31-35 CAG repeats (Semaka et al., 2013b).

The prevalence of IAs in subjects of the general population (N = 63) of Cuba was 3.9%, lower than in Europe (5.8%) (Raskin et al., 2000; Semaka et al., 2013b; Vázquez-Mojena et al., 2013). In the study with the general population of Cuba, it was observed 17 different CAG alleles, with 12-31 repeats (mean 18.3 ± 3.54). Alleles with greater frequency in this population were 16 (23%) and 17 (19.8%) CAG repeats (Vázquez-Mojena et al., 2013).

According to Paradisi et al. (2008), in the general population of Venezuela, the estimated frequency of IA carriers was less than 0.05% in 279 individuals with family history of HD. In this study, the allele size in control subjects ranged from 11 to 31 CAG repeats. The alleles with higher frequencies were those showing 17 (21.3%), 18 (16.3%), and 19 (15.0%) CAG repeats. On the other hand, the size of expanded alleles ranged from 35 to 112 CAG repeats: the alleles with 41 (14.4%), 42 (10.1%), and 43 (10.1%) CAG repeats were the most frequent ones (Paradisi et al., 2008). As mentioned earlier in this paper, Nance et al. (1999) showed a CAG expansion of approximately 250 repeats in a patient with juvenile HD.

Regarding haplogroups and IAs in South Africa, it was observed that individuals with IA with family history of HD belonged to haplogroup C (Baine et al., 2013). Furthermore, other article reports the investigation of haplotypes A, B, and C carried out in the general population of Thailand, where it was observed that individuals with IAs had haplogroups not associated with HD such as A (variant A5 and haplogroup C). In this study, the CAG allele in normal individuals ranged from 9 to 24 repeats with mean value of 16.49 ± 1.74 (Pulkes et al., 2014).

The haplotype analysis of individuals who bore IAs in the general population from Europe revealed that of 49 subjects 30 were IA carriers (61%) and their haplotypes were A1

and A2. The most frequent haplotype profiles (A1 and A2) in the general population were also evidenced in 71.1% of 135 patients with family history of HD. The most frequent normal allele seen in European population had 17 CAG repeats (Semaka et al., 2013b).

Some authors analyzed the instability (contraction or expansion) of the IA transmission to offspring. One European study analyzed prenatal tests (between 1998 and 2008) of couples with family history of HD and reported a frequency of 4% IA (van Rij et al., 2014). Another study examined 13 transmissions of intermediate and reduced penetrance alleles in 12 families from Portugal and showed only instability of transmission in 1/3 of the carriers who had reduced penetrance alleles (Sequeiros et al., 2010).

Instability of IA transmission was described in one Mexican cohort. Expansion of an IA to the complete penetrant allele was observed in two cases: one father bearing 34 CAG repeats transmitted an allele with 45 repeats to his child, as well as a father with 35 repeats transmitted an allele with 71 repeats. In this study the average of CAG repeats was 19.04 among the normal alleles and 47.16 among the expanded ones (Alonso et al., 2009).

Two studies (N = 50 each) investigated clinical signs such as motor, cognitive, and behavioral impairments in individuals who bore IAs with family history of HD in comparison with a healthy control group. These studies show statistically significant difference in symptoms between the two groups. In one study the authors suggested that behavioral, motor, and cognitive changes are mild clinical manifestations of HD but relevant in patients with IA (Ha et al., 2012; Killoran et al., 2013). The same authors suggested that this behavioral phenotype may represent a prodromal phase of HD with potential subsequent clinical manifestations (Killoran et al., 2013).

DISCUSSION

The HD prevalence in different populations is the consequence of the balance between the emergence of new mutations from the IAs and the decrease of transmission of expanded alleles. In juvenile HD cases, the patient has extra-large CAG repeats (>60) and the age of onset occurs very early, decreasing the risk of transmitting the expanded allele to the next generation. Genetic counseling can also favor the decrease in HD prevalence (Warby et al., 2009).

Some authors believe that the true prevalence of HD within a population may be underestimated by up to 80% (Rawlins, 2010; Spinney, 2010). According to Hitt (2010), in a related press conference, Professor Rawlins noted that the reasons for the underestimated prevalence of HD may be 2-fold. One reason is that it was not possible, until recently, to test for the presence of HD, and so it may be misdiagnosed as some other disorders. The second reason is that people and families often hide the fact that they have HD. Other authors have also reported this difficulty for calculating HD prevalence in a Brazilian town that has a cluster of HD (Hitt, 2010; Agostinho et al., 2015).

New mutations for HD are more common than expected. Approximately 10% of individuals who develop the clinical disease have parents who, based on their own phenotype, would not have been considered as carriers of an HD mutation; however, they were carriers of IAs (Falush et al., 2001).

The IA frequency was relatively common in the general population of North America, often ranging between 1.0 and 3.9% (The Huntington's Disease Collaborative Research Group, 1993; Semaka et al., 2006).

According to Goldberg et al. (1995), in a study performed in Canada, the IAs found in the general population occurred with a frequency of 1.9%. In another study, the IA frequency found in Europe was 0.75% (12/1595 control chromosomes) (Kremer et al., 1994; Goldberg et al., 1995). Goldberg et al. (1995) classified as IAs the alleles bearing between 29 and 35 CAG repeats. On the other hand, Kremer et al. (1994) classified as IAs those bearing 30-35 CAG repeats and Lima e Silva et al. (2000) classified as IAs those showing 35-40 CAG repeats.

It is known that an IA is defined as the smallest number of CAG repeats that can expand to a mutant HD allele when transmitted to the next generation. The lower range of IAs was set when a case report was published showing a paternal expansion of an allele with 27 CAG repeats transmitted to his son who was the first symptomatic individual in the family. The upper limit of the IA was defined when it was shown that individuals who bore 36 CAG repeats could have HD symptoms (Kremer et al., 1994; Kelly et al., 1999; Semaka et al., 2006).

It is important to make standardization defining the number of CAG repeats for IAs because the frequency of IAs may contribute to the rise of new HD cases, and, therefore, can be relevant for epidemiological studies (Warby et al., 2009).

Previous studies have shown a significant correlation between the numbers of CAG repeats in normal chromosomes and the prevalence of HD. The number of CAG repeats in normal chromosomes is significantly higher in populations presenting high prevalence of HD (Kremer et al., 1994; Squitieri et al., 1994). Higher averages of CAG numbers in normal chromosomes were found in Mexico, Cuba, Brazil, Europe, North America, and Oceania (Raskin et al., 2000; Wexler et al., 2004; McNicoll et al., 2008; Alonso et al., 2009; Semaka et al., 2013b; Vázquez-Mojena et al., 2013).

It was observed that 33% (41/124) IAs bearing 30-35 CAG repeats had shown instability when transmitted to the next generation; on the other hand, a lower frequency with 23% (13/57) IAs bearing 27-29 CAG repeats was transmitted with variation. The authors suggest that until we understand the clinical implications of HD alleles with 27-35 CAG repeats and establish reliable risks of instability, we should exercise caution when translating these results to the clinic (Semaka et al., 2010).

The vast majority of CAG-expanded chromosomes are found only in specific haplogroup variants (A1 and A2). Both longer-normal (20-26 CAG) and intermediate allele (27-35 CAG) chromosomes are specifically enriched for the same haplogroups as the HD patients. Less unstable IAs are found because they are classified as haplogroup C on which CAG expansion almost never occurs. CAG expansion occurs preferentially in specific haplotypes (Goldberg et al., 1995; Warby et al., 2009).

Studies that had examined the degree of instability of IA familial transmission have curious findings (Brocklebank et al., 2009; Semaka et al., 2010). In a Venezuelan family, no *de novo* mutation was documented within 69 transmissions of IAs (Brocklebank et al., 2009). On the other hand, 14% of the same kind of transmissions led to expansions, in future generations, in 51 families from Northern Europe (Semaka et al., 2010).

Some authors compared the intergenerational instability between the IAs in the general population and in HD families. They concluded that the IAs originated from individuals with HD history were significantly more unstable than those IAs found in the general population. However, for both cases, changes in the allele size during transmission were small, and there was a sporadic risk of inheriting HD expanded alleles. These sporadic cases of HD (4.5%) came from individuals harboring IAs who had family history of HD, not being reported in offspring of those harboring IAs from the general population (Squitieri et al., 1994; Goldberg

et al., 1995; Chong et al., 1997; Warby et al., 2009; Semaka et al., 2013a,b). One explanation for this should probably be their haplogroup.

CONCLUSION

It is clear that the larger is the number of IAs in the population, the greater will be the incidence of HD. Although, IAs can be found in general population as well as in the HD families, we cannot say which group has the higher frequency of IAs; since it is not usual to investigate statistically significant samples to draw valid inferences for general population and HD families for comparison. It is important to investigate the haplotype background of these IAs, taking into consideration the set of SNPs that determine the haplogroups, because not all IAs have the same predisposition to vary the number of CAG repeats.

The variation of prevalence rates in different places around the world, in addition to HD clinical variability are factors that increase the complexity in conducting epidemiological surveys of the disease. It is worth mentioning the importance of using the classification of HD alleles, by repeat length, according to the ACMG/ASHG statement. This could facilitate data sharing and discussion among different authors worldwide. Therefore, the standardized classification of CAG alleles according to its size would reduce disparate studies about the disease, which would lead to more reliable epidemiological studies. Furthermore, to determine the actual frequency of IAs in the general population and the number of symptomatic patients would be crucial for genetic counseling.

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