Chapter 1

Genetic Modifiers of CAG.CTG Repeat Instability in Huntington's Disease Mouse Models

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http://dx.doi.org/10.5772/66438

Abstract

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder whose characterstics were first described by George Huntington in 1872. Several decades later, in 1993, the mutation behind this disease was found to be an unstable expanded CAG repeat within exon 1 of the *HTT* gene localized on the short arm of chromosome 4. The majority of HD patients carry more than 40 CAG repeats, which become unstable and usually increase in size in successive generations and in tissues. In order to dissect the molecular mechanisms underlying CAG repeat instability, several HD mouse models have been created in the 1990s. Significant data have revealed that the absence of proteins from the mismatch repair (MMR) or the base and nucleotide excision repair decreased the pathogenic expansion-biased somatic mosaicism and/or intergenerational expansions. Some polymorphic variants of MMR genes have also been associated with reduced somatic expansions. Since expansion-biased somatic mosaicism likely contributes to disease manifestations, these results suggest that genetic modifiers of instability may also affect disease severity. In this chapter, we provide an overview of the data recently published about DNA instability; the roles of genetic modifiers of trinucleotide repeat dynamics in mouse models; and the possible therapeutic interventions.

Keywords: Huntington disease, DNA instability, mouse models, genetic modifiers, MMR

1. Introduction

Expansions of repetitive DNA sequences, including trinucleotide repeats, are associated with a large number of neurological and neuromuscular disorders, such as fragile X syndrome,

myotonic dystrophy type 1 and Huntington's disease (HD) [1, 2]. In the healthy population, the triplet repeat tract size varies between 5 and 30 repeats and is stable. In HD patients, the pathogenic allele contains more than 40 repeats and becomes highly unstable and usually increases in size in successive generations (intergenerational instability) and in somatic tissues (somatic instability). Longer expanded alleles are associated with more severe forms of disease and result in a decreasing age of onset from one generation to the next [1, 3, 4]. Among trinucleotide repeat disorders, HD disease is the fourth reported.

1.1. Clinical picture of HD

Huntington's disease is an autosomal dominant neurodegenerative disorder with a worldwide incidence varying from 0.1 to 10 per 100,000 people depending on the country. The estimation of prevalence varies according to haplogroups studied: it is estimated from 2 to 7 per 100,000 in the Caucasians and only 0.1–1 per 100,000 in Asians and Africans [5, 6]. Adult-onset Huntington disease is the most common form of HD and usually presents in early middle life. HD symptoms include uncontrolled movements such as chorea, progressive cognitive impairment and neuropsychiatric manifestations. The rare early-onset form of the disease also called juvenile form presents more severe symptoms with rigidity and motor dysfunctions [7]. HD symptoms and severity vary greatly among family patients and between juvenile and adult onset forms. Currently, no treatment is suitable to stop or reverse any form of HD.

1.2. Genetic of HD

HD is caused by an unstable expanded CAG repeat within exon 1 of the *huntingtin* (*HTT*) gene also called *HD* or *IT15* that localizes on the short arm of chromosome four, 4p16.3 [8]. The normal *HTT* gene contains from 5 to 35 stable CAG repeats, while the majority of HD patients have expanded repeats of above 40 CAG units that are fully penetrant. In rare cases, HD symptoms are associated with small CAG repeats from 36 to 39 CAG, which show low penetrance [9, 10]. Abnormal CAG repeat tracts become unstable in the germline, with a striking tendency toward expansions. Because longer alleles are associated with more severe form of HD, expansion-biased intergenerational instability results in a decreasing age of onset from one generation to the next, a phenomenon known as anticipation. Typically, 40–50 CAG repeats correlate with later-onset of HD, whereas a mutation greater than 50 CAG repeats results in a juvenile form. Two large analyses in HD patients (360 and 440 individuals, respectively) have reported a high negative correlation between the disease age of onset and the inherited CAG repeat length [11, 12]. Intergenerational instability biased toward expansions provides the molecular basis for clinical anticipation observed in HD (**Figure 1**).

1.2.1. Intergenerational instability

The frequencies of expanded, unchanged and contracted alleles have been investigated by directly comparing the length of the repeat tract in each parent with that is observed in their progeny to estimate the degree of intergenerational instability in each set of HD cohort. Small normal alleles with CAG repeat size ranging from 10 to 28 CAG are genetically stable with germline mutation rates <1% per generation [13]. However, the mutation frequency rises

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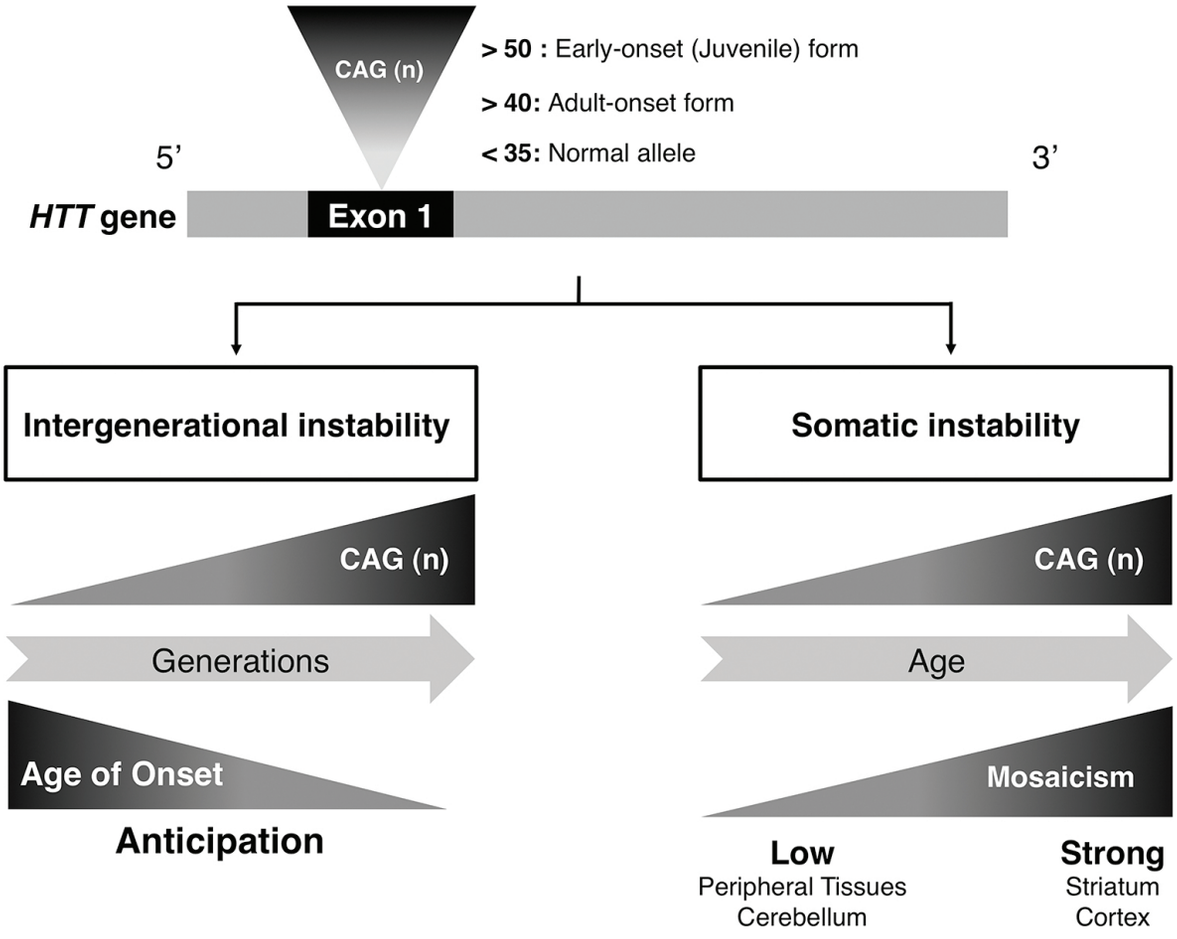

Figure 1. CAG repeat dynamics in HD: features and implications of intergenerational and somatic instabilities.

dramatically with the increasing size of the allele. Indeed, a CAG repeat size change on expanded allele in the range of 36–49 repeats occurs in >70% of transmissions from affected parents to HD children. A similar rate of expansion was found between multiethnic cohorts [13–19]. In the two largest cohorts (>250 parent-offspring pairs), the frequency of expansions was estimated to be 52.1% in a multiethnic HD population and 67.3% in the Dutch cohort, whereas only 18.1% and 25.2% contractions were observed, respectively [13, 18]. For individuals carrying more than 49 CAG repeats, the mutation rates go up to >95% per generation [14, 20]. In all cases, the frequency of expansions always exceeds the frequency of contractions in HD populations. The instability of the CAG repeat between generations depends on the sex of the transmitting parent and the length of the repeat itself. Studies of the two cohorts of HD individuals with the mean size of ~43 CAG repeats have shown that 61–68% of paternal transmissions resulted in expansions, whereas the majority (>60%) of maternal transmissions resulted in contractions or CAG stabilization [13, 18]. The largest expansions, associated with the juvenile form of HD, are almost observed in male transmissions and are influenced by the CAG repeat length of the transmitting parent [13]. The largest HD cohort study (337 transmissions) has shown that the age of the transmitting parents and the sex of offspring do not affect the intergenerational instability, suggesting that the gender of affected parents is the

major modifier of intergenerational instability [18]. Repeat size variability has been investigated in spermatogonia, postmitotic spermatid and matura spermatozoa collected by laser capture microdissection of testis from two HD patients in order to determine the timing of repeat instability. Interestingly, CAG repeat expansions were already present before the end of the first meiotic division and the frequency continues to increase in postmeiotic cell population suggesting that the primary source of instability occurs in spermatogonia [21].

1.2.2. Somatic instability

Several studies have reported that the expanded CAG repeat allele is also unstable in somatic tissues and increases in length over time [22–25]. Somatic CAG repeat size variation was analyzed by bulk PCR in each tissue whereas the degree of somatic mosaicism was quantified by a more sensitive PCR-based approach called Small-Pool PCR [26]. This method allows to accurately assess the variation of CAG repeat length of each HD expanded allele in tissues, using successive DNA dilutions in order to amplify few template molecules per reaction (**Figure 2A**). The dynamics of somatic CAG repeat instability varies between and within tissues with the highest instability observed in the striatum and cortex, two tissues that show the most pronounced neuropathological abnormalities [22, 23, 25]. In a large Venezuelan HD cohort, a positive correlation was reported between the size of progenitor alleles (inherited alleles) and the expansion-biased somatic mosaicism in buccal cells from individuals at the same age. This observation suggests that the size of the inherited CAG repeat is an important modulator of somatic instability [27]. Furthermore, it has been reported that CAG repeat expansion length in the cortex is associated with an earlier age of disease onset suggesting that somatic instability is a significant predictor of the age of onset [28]. Interestingly, somatic instability was not observed in two fetuses at 12–13 weeks suggesting that the somatic expansion event occurs later in the stages of fetal development or from birth throughout the patient's life [29].

Together, these data have clearly demonstrated the contribution of the sex of the transmitting parent and the inherited length of the CAG repeat in the dynamics of intergenerational and somatic instability in HD patients. Moreover, both germline and somatic mosaicism level seems to be linked to the disease onset and to the progression of HD symptoms. Thus, aiming at decreasing the size of expanded alleles or the level of somatic mosaicism would be an attractive therapeutic strategy. In the majority of analyses, the degree of expansion length variability between tissues and individuals cannot be explained only by the age, sex of the transmitting parent and the progenitor allele size, therefore implying that genetic factors might influence either germline or somatic instability. In 2012, the study of a large Portuguese HD cohort has reported some HD families with extreme repeat length changes from parents to offspring suggesting the existence of modifiers that may be heritable [19]. Hence, the understanding of CAG repeat instability is crucial to improve the therapeutic possibilities. Analyses of genetic modifiers of instability and dissection of mechanisms involved in this process are compromised by the limited accessibility of human samples and clinical information. Then, knockout, transgenic and knock-in HD mouse models have been generated to dissect the molecular mechanisms of instability and the pathogenesis of HD disease [30, 31].

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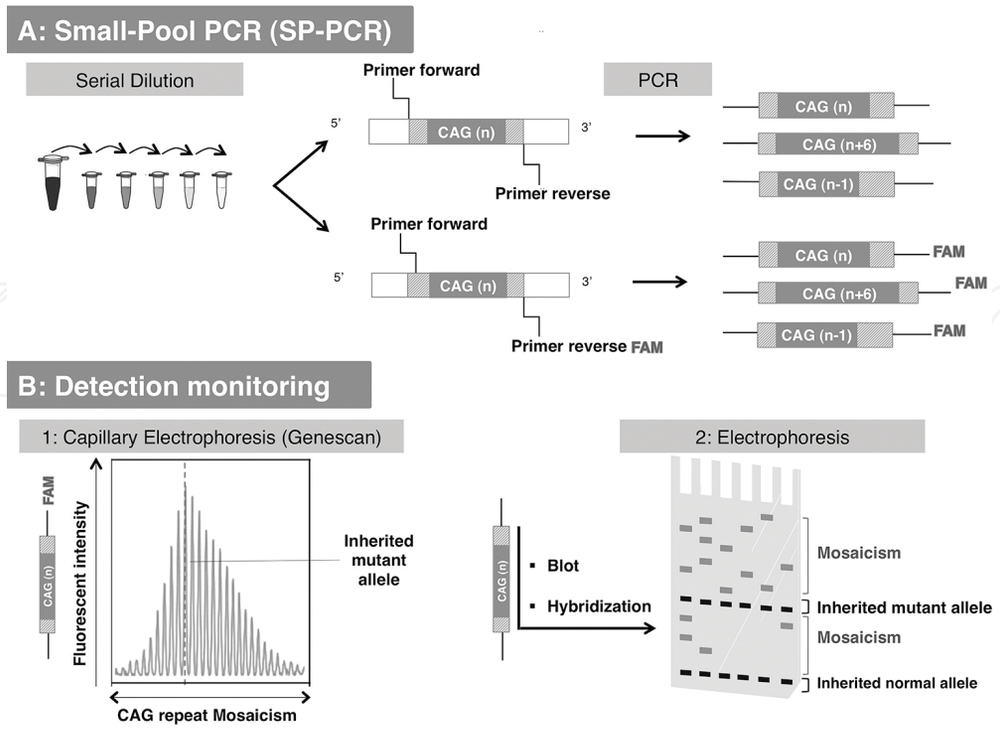

Figure 2. Methods to analyze CAG repeat length in germline and somatic tissues.

2. Mouse models of CAG repeat instability

The dynamics of expanded CAG repeat has already been analyzed in different simple organism strains such as bacteria and yeast by inserting a plasmid with a pathogenic CAG repeat. Analyses in *E. coli* and *S. cerevisiae* have provided valuable insight into factors affecting the CAG repeat instability. However, these organisms displayed a CAG repeat instability biased toward contractions in clear contrast to HD patients. Furthermore, both these organisms differ from mammals by cellular processes such as DNA repair and replication pathways. Therefore, mouse models have been generated to identify genetic modifiers of instability and to specify the mechanisms by which they act in HD. These mouse models including two HD transgenic mice with short gene fragment or BAC (R6 and BACHD), eight knock-in (the HdhQ20, HdhQ50, HdhQ92, HdhQ111, Hdh4/80, or Hdh6/72 lines, HdhQ150 and HdhQ80), have been created to analyze the dynamic of CAG repeat instability in germline and somatic tissues by different methods [24, 32–36] (**Table 1**). The first method determines CAG repeat size by using unlabeled primers flanking CAG repeat. PCR and SP-PCR products can be resolved on agarose gel with internal size standards and detected with radioactive probes [37]. The second method measures the length of CAG tracts by using primers flanking CAG repeat expansions, labeled with the 5-carboxyfluoroscein fluorochrome. PCR products are electrophoresed/separated in an automated sequencer together with internal size standards. In this case, the sizing of the PCR fragment is determined using GeneMapper software that represents the PCR fragments by peaks with single repeat unit resolution (**Figure 2**).

	Mouse models	Genetic background	Transgene	CAG repeat length	Mutation rate	Intergenerational instability (CAG length variation)	Somatic instability (partial list)	References
Transgenic mice	**BACHD**	FVB	Human *HTT* locus	97	None	None	None	[34]
	R6 (Excluded R6/T)	CBA/C57BL/6	Human *HTT* exon 1	>110	65–84%		Striatum> kidney> cerebellum	[32, 45, 46, 51, 52]
Knock-in mice	**Hdh^{Q80}**	C57BL6/J		80	~20%	↑ expansions (male transmissions)		[35]
	Hdh4/^{Q80}	129Svter/ C57BL6		80	~20%	↓ contractions (female transmissions)	Striatum> cerebellum> liver	[25, 36]
	Hdh6/^{Q72}			72	~20%			
	Hdh^{Q50}			48	4%	Low	None	[54]
	Hdh^{Q20}		CAG repeat locus	18	None	None		[24]
	Hdh^{Q92}	129SvEv/CD1		90	49%	↑ expansions (male transmissions)	Striatum> kidney> cerebellum	[24, 56, 57]
	Hdh^{Q111}			109	73%	↓ contractions (female transmissions)		[24, 56, 57, 58]
	Hdh^{Q150}	C57BL6/129Ola		150	16%	ND	Striatum> olfactory bulb> cerebellum	[25, 33, 38, 39]

Table 1. HD mouse models of CAG repeat instability.

BACHD mouse model was established by the introduction of a full-length human *htt* locus containing exon 1 with 97 mixed CAA-CAG repeats in the *FVB* background. These mice do not exhibit any repeat instability or contraction in germline and in brain tissues at 12 month of age [34]. The stability of CAG triplet repeat results from the CAA interruption within the CAG repeat tract, which probably modifies the DNA structure and then the repeat dynamics [1]. Compared to BACHD mice, HdhQ150 knock-in mice were generated by replacement of the murine short CAG repeat in exon 1 with a 150 CAG repeat expansion in a mixed C57BL/6/129Ola genetic background [33]. HdhQ150 animals reproduce somatic mosaicism in different brain regions, most particularly in the striatum like HD patients [25, 38]. HdhQ150 mice displayed some HD symptoms that seem more severe in homozygous mice [33, 39, 40]. Recently, heterozygous hdhQ250 mice have been generated from hdhQ150 by selective breeding and shown more severe neurological symptoms than heterozygous hdhQ150 mice [41]. Hdh4/Q80 and Hdh6/Q72 mice have also been obtained by replacement of short CAG repeat with 72 or 80 CAG repeat expansions in *htt* murine gene context. Both these lines have shown intergenerational instability biased toward expansions in paternal transmissions and contractions in maternal transmissions like in HD patients. However, the mutation frequency is only 20% across generations compared to 70% in HD individuals [36]. Hdh4/Q80 and Hdh6/

Q72 have displayed somatic mosaicism that is tissue-specific, age-dependent and CAG repeat length dependent [25, 36, 42, 43]. Some neurological and motor impairment have also been described in these mice and might be correlated to the somatic mosaicism level [25, 36, 44]. A different HdhQ80 mouse model has been created by replacement of the murin exon 1 with the human exon 1 carrying ~80 CAG repeat using C57BL6/J mice. Small expansions upon paternal transmissions and CAG repeat contractions across maternal transmissions have been reported in about 20% of cases. As observed in HdhQ150, Hdh4/Q80 and Hdh6/Q72, HdhQ80 mice have shown somatic mosaicism that is age-dependent and biased toward expansions with the highest levels in the striatum and liver [35]. Compared to BACHD and knock-in mice described above, R6 and HdhQ111 mice are the most commonly used to identify the genetic modifiers of CAG repeat instability [24, 32, 45]. Therefore, we will review the somatic and intergenerational instability features for both these animal models in the next section.

2.1. R6 transgenic mouse lines

The first successful HD transgenic mouse model was created in 1996 and called R6 lines of HD transgenic mice [32]. These mice were obtained by random integration of a short 5′ fragment of human *HTT* gene containing 1000 bp of 5′UTR, exon 1 with ~130 CAG repeat tracts and the beginning of intron 1 in a CBA/C57BL6 genetic background. Five lines of mice were obtained with different insertion sites and CAG repeat lengths. The R6/T line carries a truncated HD transgene without CAG repeat expansions, the R6/0 line carries 142 CAG repeats, R6/1 carries 116 repeats, the R6/2 carries 144 CAG and the R6/5 line carries multiple copies of transgene with 128, 132, 135, 137 and 156 CAG repeats, respectively. R6/0 mice have shown no transgene expression and no phenotype compared to R6/1, R6/2 and R6/5. These three mouse lines develop progressively neurological abnormalities and show a variable age of onset that depends on the CAG repeat length and on the transgene expression levels. R6/1 and R6/2 are the most studied of these lines to assess both HD pathogenesis and CAG repeat instability.

To evaluate intergenerational CAG repeat lengths, fluorescent PCR using DNA from tail biopsy at 3 weeks of age was performed in R6/0, R6/1, R6/2 parents and offspring. The comparison of CAG repeat lengths between parents and their progeny is limited in R6/5 mice due to the integration of multiple transgene copies in the genome of this line. Compared to R6/1 and R6/2, R6/0 mice do not show any evidence of CAG repeat instability and any transgene expression. As observed in HD patients, R6/1 and R6/2 mice mimic intergenerational instability biased toward expansions across paternal transmissions and toward contractions during R6/1 maternal transmissions (R6/2 female mice are infertile) with a mutation rate from 65 to 84% [45, 46]. Interestingly, the CAG repeat size changes depend on the gender of R6/1 embryos with a high expansion rate in males and high contraction rate in females from the same fathers suggesting that offspring sex-dependent genes modulate intergenerational instability in R6/1 mice [46]. In R6/2 mice, the size of transmitted CAG expansion increases with the age of transmitting males [45]. A selective R6/2 breeding enabled to obtain numerous R6/2 colonies with inheriting CAG repeat ranging from ~110 to 450 [47–49]. The size of CAG repeat is positively correlated with the severity of symptoms up to ~160 CAG repeats [47]. Surprisingly, some neurological symptoms and a lifespan are greatly ameliorated in R6/2 mice

carrying more than 200 CAG repeat expansions [47–49]. These unexpected results can be explained by transgene expression decrease observed in these mice [48]. A spontaneous contraction from 116 to ~89 CAG repeat was described in R6/1 mice [50]. These mice showed a decreased age of onset and a HD phenotypic improvement compared to R6/1 mice with 116 CAG repeat supporting the relationship between the CAG repeat size and the progression of symptoms.

Somatic instability of the CAG repeat tracts has been also reported in R6 lines carrying CAG repeat expansions excepted for the R6/0 line [45]. R6/1 and R6/2 recreated expansion-biased, age-dependent and tissue-specific somatic mosaicism as observed in HD patients [38, 51, 52]. Liver and striatum have shown the highest levels of instability biased toward expansions compared to other tissues that have shown low or no instability in both lines. Two distinct modes of somatic expansion have been described in tissues from R6/1 mice. Striatum and cortex have shown a periodic expansion, whereas the other tissues reproduce a short continuous expansion overtime suggesting different mechanisms of instability in these tissues [51]. Large spontaneous expansions (>200 CAG) have been described in striatum and cortex from R6/2 mice [52] consistent with the observations done in brain from HD patients [25, 43]. In R6/2 mice, the somatic mosaicism is correlated with the transmitted CAG repeat size but the somatic variation is not linear, particularly in striatum [52]. Interestingly, the frequency of CAG contractions increases in brain tissues and liver from mice with more than 500 CAG repeats [52] and could also explain the progressive reduction of neurological symptoms and prolonged lifespan in R6/2 mice with >200 CAG repeats [47–49]. Somatic instability has been noticed in dividing cells suggesting a role of DNA replication in the dynamic of triplet repeat instability. However, an increase of CAG repeat length has also been reported in terminally differentiated neurons from R6/1 mice suggesting the role of cellular processes independent of DNA replication in the somatic mosaicism [38]. Recently, an effect of mouse genetic backgrounds on the dynamics of CAG expansions has been reported in tissues from R6/1 mice with high CAG somatic mosaicism on a B6 background and low level in BALB/cBy backgrounds suggesting the existence of genetic modifiers of instability [53].

2.2. HdhQ92-111 mouse models

The first knock-in mice called HdhQ50 have been generated in 1997 using homologous recombination in ES cells to replace short murine CAG repeat by 48 CAG repeats in 129SvEv/CD1 mice [54]. In 1999, three other knock-in mouse models (HdhQ20, HdhQ92 and HdhQ111) using the same strategy have been generated with 18, 90 and 109 CAG repeat tracts, respectively [24]. These four knock-in mice share the identical murine genomic environment (91% of similarities with *HTT* human) and differ only by the size of CAG repeat length. Knock-in mice with >50 CAG repeats reproduce the pattern of intergenerational instability observed in HD patients. The mutation rate is only 4% in HdhQ50, 49% in HdhQ92 and 73% in the HdhQ111 supporting that intergenerational instability depends on CAG repeat length as described in R6 mice [24]. However, no age effect has been observed in these knock-in mice compared to R6/2 [24]. These divergent results could be explained by the CAG repeat genomic context and the genetic background. Interestingly, an effect of mouse genetic backgrounds on mutation

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rate and range of CAG repeat length changes upon male transmission was reported in HdhQ111 supporting the role of genetic modifiers on CAG instability process [55].

Somatic CAG repeat variations have been observed in HdhQ92 and HdhQ111 mice in brain and some peripheral tissues with the highest accumulation of expansions in striatum and liver [24, 56, 57]. Both these tissues showed a bimodal distribution of repeat lengths compared to spleen and tail that showed a unimodal distribution [56]. CAG expanded alleles were broadly distributed in striatum compared to liver that showed distinct populations of CAG repeat expansions [56]. Somatic instability depends on the CAG repeat size and the age of animals and is tissue-specific as reported in R6 mice [24, 56, 57]. The relationship between somatic mosaicism and HD phenotype remains unclear but some data have reported that somatic mosaicism is not correlated with the initiation of disease but may be correlated with the progression of HD phenotypes [57, 58].

In conclusion, HD mouse models closely reproduced the dynamic of instability observed in HD patients. Intergenerational instability is biased toward expansions and depends on the CAG repeat length and the sex of transmitting parent. HD transgenic and knock-in mouse models also mimic the somatic instability of HD patients, with the highest somatic mosaicism in the striatum that is the most affected tissue in HD. Some differences in the dynamics of intergenerational instability between HD patients and HD mouse models can be noticed. Despite a high level of instability biased toward expansions in paternal transmissions and contractions in maternal transmissions in both species, the critical CAG repeat threshold length differs between human and mice corresponding to 35 CAG repeats in human and more than 80 CAG repeats in mice. Moreover, no spontaneous large CAG repeat expansion has been observed in HD mouse models during paternal transmissions, in contrast to HD patients. These differences may be explained by genetic and environmental factors. Despite these divergences, the development of HD mouse models provided a powerful tool to explore trinucleotide repeat dynamics. Several data have suggested that the size, sex and the age factors are not sufficient to explain the level of meiotic and mitotic instabilities observed in HD patients and mice supporting the contribution of genetic modifiers in CAG repeat instability processes. Among described mouse models, R6 and HdhQ111 were commonly used to investigate the role of genetic modifiers on the level of intergenerational and somatic instability in HD.

3. Genetic modifiers of CAG repeat instability

The absence of correlation between CAG repeat somatic mosaicisms and the corresponding tissue proliferative rates and the destabilization of CAG repeat in murine mature neurons support the involvement of DNA repair pathways in the CAG repeat instability processes (**Table 2**). To identify the DNA repair pathways involved in the germline and somatic CAG repeat instability, R6/1 or HdhQ111 mice were crossed with mouse lines deficient for individual DNA repair genes. CAG repeat length changes upon transmissions were determined by comparing the CAG repeat size in the HD transmitting mice with CAG repeat length in the HD progeny for each DNA repair genotype (+/+ to +/+ and -/- to -/- and/or +/- to +/-). Further-

more, different methods have been described to quantify the degree of somatic instability and have made it possible to compare the level of somatic mosaicism between HD mice mutated and not for DNA repair genes [53, 57, 59–61].

Gene modifiers	DNA repair systems	Gene status	Mouse models	Effect on CAG repeat length		References
				Intergenerational instability	Somatic instability	
Msh2	MMR	KO	Hdh^{Q111}	↓ expansions ↑ contractions (male transmissions)	CAG repeat stabilization	[58, 67]
				No change (female transmissions)		
			R6/1	No expansion (male transmissions) ND (female transmissions)	↓ expansions	[59, 63]
Msh3			Hdh^{Q111}	No significant change	CAG repeat stabilization	[64]
		C57BL/6J	R6/1	ND		[53]
		BALB/cByJ				
Msh6		KO	Hdh^{Q111}	No change (*Msh6-/-*)	No change	[64]
Mlh1				ND	↓ expansions	[69]
Mlh3						
Ogg1	BER		R6/1	No change	↓ expansions	[65, 70]
Neil1				↓ expansions (male transmissions)		[61]
Fen1				ND (female transmissions)	No change	[66]
Csb	NER			↑ expansions ↓ contractions		[65]
Xpc				No change		[64]

ND, not determined.

Table 2. DNA repair genetic modifiers involved in CAG repeat instability in mouse models.

3.1. Genetic modifiers of intergenerational instability

Despite some controversial results, the analyses in *E. coli* and *S. cerevisiae* suggested an effect of mismatch repair (MMR) proteins on the dynamics of CAG repeat instability. MMR proteins preserve genome integrity by repairing erroneous insertion, deletion and misincorporation of bases that occur during replication and escape proofreading. Two MutS heterodimeres, MutSα (MSH2-MSH6) and MutSβ (MSH2-MSH3) recognize replication errors and recruit MutLα (MLH1-PMS1/2) and MutLγ (MLH1-MLH3) to activate the repair pathway [62]. The breeding of HD mouse models in MMR-deficient genetic backgrounds has provided insight into the mechanisms of CAG repeat instability. In R6/1 mice, *Msh2* deficiency abolishes CAG

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repeat expansion in the male germline suggesting that MSH2 promotes CAG repeat expansion (no data for maternal transmissions) [59, 63]. A further study in HdhQ111 knock-in mice revealed that the effects of *Msh2* mutation on the intergenerational dynamics seem to be more complex. The absence of two *Msh2* alleles suppresses the expansions in favor of contractions without changing the mutation rate (corresponding to expansion and contraction frequencies) in paternal transmissions [58, 64]. In contrast, a majority of contractions and a few expansions were detected in female germline transmissions in both *Msh2*+/+ and *Msh2*-/- backgrounds [24, 58]. Therefore, although MSH2 appears to be required in paternal CAG repeat expansions, the CAG repeat gains in female germline and CAG repeat contractions seem to be generated by *Msh2*-independent processes [58]. MSH2 binding partners, MSH6 or MSH3, did not alter the frequency of maternal changes, which is consistent with the lack of involvement of MSH2 in female germline. The effects of MSH3 and MSH6 on paternal transmissions of the expanded CAG repeat are more complex. The loss of *Msh6* or *Msh3* did not significantly affect the paternal mutation frequencies and the frequency of expansions and contractions. However, a shift from expansion to unchanged and contracted CAG repeat length is observed in *Msh3*-/- or *Msh3*+/- transmissions compared to *Msh3*+/+ transmissions suggesting that some paternal expansions might depend on MSH3 protein. These results together suggest that the majority of paternal expansions occur via MSH2, independently of MSH3 and MSH6 partners in HdhQ111 mice and that other DNA repair proteins are involved in CAG repeat parental expansions and contractions observed in HD mice [64].

The involvement of base excision repair (BER) and nucleotide excision repair (NER) in CAG repeat instability have been tested in R6/1 mice bred in a BER gene (*Ogg1* or *Neil1*) and NER gene (*Csb* or *Xpc*) deficient backgrounds. The loss of 7,8 dihydroxy-8-oxoguanin-DNA-glycosylase, OGG1 did not affect the dynamic of instability in the germ cells [65]. However, NEIL1, another glycosylase of BER contributed to paternal expansions in R6/1 mice. In the absence of *Neil1*, the CAG repeat tracts were more stable with a tendency toward contraction in male germline compared to *Neil1*+/+ [61]. Interestingly, an increase of CAG repeat expansions and a decrease of contractions in paternal transmissions have been observed in *Csb*-deficient mice suggesting that CSB promotes CAG repeat contractions during paternal transmissions just like MSH2 promotes expansions in HD mouse models [65]. In contrast to *Cbs* results, *Xpc* did not affect the dynamic of CAG repeat instability in R6/1 mice. It has also been reported that FEN1, an endonuclease involved in the DNA replication but also in BER intermediates, may stabilize CAG repeat in the *Fen1*+/- male germline by preventing deletions and modestly increasing expansions but the effect seems to be low [66].

In conclusion, these data have shown that MSH2 and NEIL1 proteins are involved in the formation of intergenerational repeat expansions in HD mouse models with the highest effect of MSH2, suggesting that these genes are genetic modifiers of intergenerational instability in HD. Moreover, the shift toward contractions observed in the absence of *Msh2* and *Neil1* reveals that the repeat could be processed through a distinct pathway leading to contractions via CSB or other DNA repair proteins.

3.2. Genetic modifiers of somatic mosaicism

The analysis of CAG repeat instability has revealed a relationship between the severity of HD phenotypes and the level of expansion-biased somatic mosaicism in patients and mice. Thus, HD mouse models in DNA repair deficient background have also been used to identify genetic modifiers of somatic instability. In R6/1 and HdhQ111 mice, *Msh2* deficiency was initially reported to stabilize CAG repeat expansion in somatic tissues supporting that MSH2 also drives instability toward expansions, like in germline cells [58, 59, 67].

Compared to the results obtained in *Msh6*-deficient mice, the loss of both *Msh3* alleles stabilize CAG repeat tracts in somatic tissues suggesting that MSH3 acts as an enhancer of CAG expansions-biased somatic instability but not MSH6 [53, 64, 68]. Interestingly, the absence of one allele of *Msh3* is sufficient to decrease the somatic mosaicism in the striatum in contrast to *Msh2* supporting the idea that MSH3 levels modulate the degree of somatic instability and the progression of HD disease [64]. Various degree of repeat instability in different HdhQ111 and R6/1 mouse strains harboring the identical CAG repeat length suggest the existence of other candidate factors as a source for strain-specific variation in CAG repeat pattern [53, 55]. Interestingly, CAG repeat somatic mosaicism has been associated with *Msh3* polymorphisms and the level of MSH3 protein [53]. It has been reported that expansion changes were higher in striatum and liver from R6/1 mice carrying the homozygous B6 *Msh3* gene on a CBy genetic background than mice carrying the homozygous CBy *Msh3* gene on a B6 genetic background (mice obtained by selective breeding). The loss of one B6 *Msh3* allele in mice on a CBy genetic background was sufficient to decrease CAG repeat instability, consistent with the results obtained in *Msh3*-deficient mice [53]. Thus, naturally occurring MSH3 protein polymorphisms modify the dynamic of CAG repeat instability in mice and could modulate HD pathogenesis in humans. Together, these data have shown that MSH2 and MSH3 proteins are strongly required in the generation of somatic expansions.

To identify other genetic modifiers of CAG repeat instability, linkage analyses have been performed in different HdhQ111 strains that showed CAG repeat instability variation [69]. A single quantitative trait locus on chromosome 9 and particularly in MutL homolog *Mlh1* gene has been identified and associated with CAG repeat instability. Then, somatic instability has been quantified in B6 HDHQ111 mice in the absence of one or two *Mlh1* alleles. Although one functional *Mlh1* allele was still sufficient to generate high levels of repeat expansion, the loss of both *Mlh1* alleles abolished CAG repeat expansion in striatum suggesting that MLH1 was required in somatic expansion. A second MutL homolog has been shown to act as an enhancer of CAG repeat expansions. Indeed, expansion-biased somatic mosaicism is reduced in *Mlh3* heterozygous knockout mice and totally abolished in *Mlh3* homozygous knockout mice suggesting that MLH3 is a limiting factor on the process of expansion as reported for MSH3 protein [69].

Other DNA repair systems, such as BER and NER have also been investigated in R6/1 mice to understand the somatic expansion variation observed between and within tissues. A loss of *Ogg1* suppressed CAG somatic expansions in 70% of R6/1 mice. The same study has reported that OGG1 initiated age-dependent CAG repeat expansion mice, suggesting that age-dependent somatic expansion associated with HD occurs in the process of removing

http://dx.doi.org/10.5772/66438

oxidized base lesions [70]. Deletion of *Neil1* also reduced somatic expansions in male and female R6/1 mice with a higher effect in different brain regions from male mice [61]. In contrast to the results obtained in male germline, the absence of *Csb* and *Fen1* did not affect the dynamic of somatic instability in tissues suggesting that the role of *Csb* is specific of paternal contractions [65] and that *Fen1* partially contributes to CAG repeat expansion upon parental transmissions [66].

In conclusion, MSH2 and MSH3, partner proteins in the MutSβ MMR complex and MutLγ (MLH1-MLH3) are essential to promote expansions in HD mouse models suggesting that MutSβ and MutLγ promote CAG expansion via the mismatch repair machinery. Furthermore, CAG repeat expansion depends only partially on OGG1, NEIL1 and FEN1 proteins suggesting that other DNA repair pathways are involved in the process of instability. Some genetic modifiers such as *Ogg1* and *Fen1* impact CAG repeat instability in either somatic or germline tissues, but not in both supporting that CAG repeat instability involves different genetic players between tissues and may occur via different mechanisms. It has also shown that the degree of somatic mosaicism appears to be modulated by *Msh3* and *Mlh1* variants in B6 mice where CAG repeat expansion levels are the highest suggesting that somatic instability variation observed in HD patients could be explained by DNA repair gene and/or protein variants. Different expression levels of MSH3 and MLH3 have been identified in mouse strains that exhibit different expansion frequencies supporting that the level of DNA repair proteins might be correlated with the degree of CAG repeat instability. Other studies also support a role for the stochiometries of DNA repair proteins in CAG repeat instability [4, 64, 71–73]. Few data have reported the role of genetic factors in CAG repeat contractions mainly observed in HD maternal transmissions and only *Csb* has been reported to promote contractions in paternal transmissions. CSB protein could act on CAG repeat contraction via BER, NER, or chromatin maintenance/remodeling activity independently of MSH2 protein.

4. Are genetic modifiers a therapeutic target?

The identification of genetic modifiers of underlying CAG repeat instability is important to uncover novel therapeutic targets to slow down somatic instability and to decrease the intergenerational expansions in favor of CAG repeat contractions to prevent the disease. It has been reported that *Msh2* alleles delay the accumulation of mutant protein and destruction of mutant huntingtin in striatum and in specific neuron type from knock-in HdhQ111 mice [58, 67]. Moreover, MLH1 also contributes to nuclear huntingtin and HD inclusion phenotypes [69]. Both data suggest that MSH2 and MLH1 may enhance the HD pathogenic process by modulating the somatic mosaicism in cooperation with MSH3 and MLH3 via the mismatch repair pathway. Among MMR proteins, MSH3 and MLH3 are currently the most promising targets to decrease CAG repeat expansions, thus delaying pathogenic process, given their minor roles in the initiation of human cancer. To date, no drug has been identified to decrease the expression of MLH3 and MSH3 protein and then the somatic instability. NEIL1 and OGG1, two glycosylases of the BER pathway partially contribute to CAG repeat expansions suggesting that oxidative base damage is responsible of some CAG repeat expansions. Antioxidants may

then decrease the expansion process. Mollersen and colleagues have suggested that several antioxidants like anthocyanin decrease CAG repeat expansion in the brain from R6/1 male mice [61]. The identification of new genetic factors involved in the formation of CAG repeat contractions and a better understanding of expansion mechanisms are essential. Novel therapies based on activating the DNA repair pathways promoting contractions might be expected to have lower risk of side effects than therapies based on inhibiting the DNA repair pathways that promote expansions.

5. Conclusion

The data summarized in this chapter have shown that *cis*-elements such as DNA sequence and transcription level, mismatch repair, base excision repair and nucleotide excision repair proteins can modulate the pathogenic expansion-biased somatic mosaicism and/or intergenerational expansions contributing to the progression of HD phenotype. Natural polymorphisms in *Msh3* and *Mlh1* genes have been associated with the degree of somatic expansions in HD mice suggesting that MMR variants are involved in the somatic mosaicism variation observed in HD patients and may modulate the disease severity and age of onset. Despite a great advance on the understanding of instability, the process remains complex. Then, further studies will be needed to assess how the various DNA repair and replication proteins collaborate all together in germline and/or somatic tissues to mediate CAG repeat expansions. Moreover, future studies will be essential to identify new factors that promote contractions in the germline and in somatic tissues, to reverse the HD expansion and to stop the disease.

Acknowledgements

The authors thank Geneviève Gourdon, Mario Gomes-Pereira and Diana Dinca for helpful comments and discussions. The authors also thank Christopher Pearson group, HD/CAG.CTG repeat colleagues, Canadian Institutes of Health Research, Imagine institute, INSERM, the Association Française contre les Myopathes (AFM) and the université Paris Descartes.

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