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Intergenerational and striatal CAG repeat instability in Huntington's disease knock-in mice involve different DNA repair genes

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ABSTRACT

Modifying the length of the Huntington's disease (HD) CAG repeat, the major determinant of age of disease onset, is an attractive therapeutic approach. To explore this we are investigating mechanisms of intergenerational and somatic *HD* CAG repeat instability. Here, we have crossed *HD* CAG knock-in mice onto backgrounds deficient in mismatch repair genes, *Msh3* and *Msh6*, to discern the effects on CAG repeat size and disease pathogenesis. We find that different mechanisms predominate in inherited and somatic instability, with Msh6 protecting against intergenerational contractions and Msh3 required both for increasing CAG length and for enhancing an early disease phenotype in striatum. Therefore, attempts to decrease inherited repeat size may entail a full understanding of Msh6 complexes, while attempts to block the age-dependent increases in CAG size in striatal neurons and to slow the disease process will require a full elucidation of Msh3 complexes and their function in CAG repeat instability.

Introduction

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by the expansion of a CAG repeat within the *HD* gene encoding huntingtin (Huntington's Disease Collaborative Research Group 1993). The *HD* CAG expansion initiates a protracted cascade of events that results initially in the degeneration of medium-spiny neurons in the striatum, motor, cognitive and psychiatric decline and eventual death (Vonsattel et al., 1985; Harper 1999). The mutant *HD* CAG repeat is remarkably unstable when transmitted to subsequent generations, with a bias towards expansion in transmissions from fathers (Duyao et al., 1993; MacDonald et al., 1993; Zuhlke et al., 1993; Telenius et al., 1994; Wheeler et al., 2007). The repeat also exhibits somatic instability, undergoing expansion particularly in the striatum (Telenius et al., 1994; Kennedy et al., 2003; Shelbourne et al., 2007; Veitch et al., 2007; Gonitel et al., 2008). Significantly, age of onset and disease severity are highly dependent on CAG length (Duyao et al., 1993; Andrew et al., 1993; Snell et al., 1993; Stine et al., 1993; Gusella et al., 1996). Therefore, as intergenerational instability alters the inherited *HD* CAG repeat length and somatic instability further alters repeat length in the target tissue, understanding the factors that influence both intergenerational and striatal instability is critical as these factors may modify the disease.

HD homologue (*Hdh*) CAG knock-in mice (White et al., 1997; Wheeler et al., 1999) that replicate the genetic mutation in HD patients provide ideal models in which to study the instability of the *HD* CAG repeat in its appropriate genomic context. We have shown that Hdh^{Q111} knock-in mice recapitulate many features of repeat instability seen in patients, namely intergenerational repeat length changes with a paternal expansion bias at frequencies seen in humans, and somatic expansion that is predominant in striatum (Lloret et al., 2006; Wheeler et al., 1999). Importantly, Hdh^{Q111} mice, exhibiting accurate expression of mutant huntingtin, also display early presymptomatic phenotypes that exhibit key features of the human disease mechanism, namely,

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dominant inheritance, CAG length and time dependence, and striatal specificity, allowing modifiers of the *HD* CAG pathogenic process to be tested (Fossale et al., 2002; Gines et al., 2006; Gines et al., 2003; Wheeler et al., 2000; Wheeler et al., 2002; Wheeler et al., 2003). Significantly, the CAG repeat length dependence of knock-in phenotypes provides the possibility of testing directly whether factors that alter repeat length also alter the pathogenic process.

We previously investigated the role of mismatch repair gene *Msh2* in *HD* CAG repeat instability and the *HD* CAG pathogenic process in Hdh^{Q111} mice (Wheeler et al., 2003). In paternal transmissions *Msh2* was required for intergenerational Hdh^{Q111} CAG repeat expansions and protected against contractions. *Msh2* was also required for striatal Hdh^{Q111} CAG repeat instability and dramatically enhanced an early histological phenotype, the accumulation or epitope-accessibility of a conformation of full-length mutant huntingtin in striatal neurons, as revealed by nuclear immunostaining with the EM48 anti-huntingtin antibody. This finding strongly suggested that striatal instability contributes to the Hdh^{Q111} pathogenic process.

Here, taking advantage of the opportunity afforded by Hdh^{Q111} mice to dissect both intergenerational and somatic instability as well as *HD* CAG pathogenesis, we have investigated Msh2's binding partners, Msh3 and Msh6 (Acharya et al., 1996), in these processes, as well as nucleotide excision repair gene *Xpc* with the aim of exploring the roles of other DNA repair pathways. We have crossed Hdh^{Q111} mice onto backgrounds deficient in either *Msh3*, *Msh6* or *Xpc* to determine whether: 1. these genes influence *HD* CAG striatal and intergenerational instability in a precise genetic model of HD, 2. the same or different mechanisms underlie striatal and intergenerational instability, and 3. these genes are modifiers of an early, dominant, CAG length-dependent phenotype, nuclear mutant huntingtin immunostaining in striatal neurons.

Materials and methods

Mice

Hdh^{Q111} knock-in mice (White et al., 1997; Wheeler et al., 1999) were maintained on a CD1 (Charles River Laboratories) background. *Msh3* and *Msh6* knockout mice (Edelmann et al., 2000; Edelmann et al., 1997) (C57BL/6J) were obtained from Dr. Winfried Edelmann and *Xpc* knockout mice (Cheo et al., 1997) (C57BL/6J) were obtained from Dr. Errol Friedberg. *Msh2* knockout mice (129Ola/FVB) were from Dr. Hein te Riele (Toft et al., 1999). $Hdh^{Q111/+}$ mice were crossed with each DNA repair knockout strain to generate mice heterozygous for each mutation. These mice were then crossed to generate $Hdh^{Q111/+}$ littermates that were wild-type (+/+), heterozygous (+/−) or homozygous mutant (−/−) for each DNA repair gene. These mice were either used for assessment of somatic effects (instability, nuclear huntingtin immunohistochemistry), or were the transmitting parents for the intergenerational instability experiments. Genetic background can modify instability and nuclear huntingtin accumulation (Lloret et al., 2006). However, assuming that such modifier genes segregate independently from the repair genes being analyzed they will occur equally in the repair gene mutants and their wild-type littermates. Thus, sufficient numbers of mice of each genotype will allow identification of effects of the repair gene mutants over and above those due to genetic background. Therefore, to minimize potential confounding effects of genetic background all genotypic comparisons for intergenerational instability, somatic instability and EM48-immunohistochemistry were performed using littermates. The number of mice we have used in each analysis was based on numbers of mice needed to detect modifier effects of the *Msh2* gene on a mixed genetic background (Wheeler et al., 2003). All analyses were with heterozygous $Hdh^{Q111/+}$ mice. All animal procedures were carried out to minimize pain and discomfort, under an approved Institutional Animal Care and Use Committee protocol.

Genotyping and HD CAG repeat length determination

Genomic DNA was isolated from tail biopsies at weaning (for routine genotyping and intergenerational instability analysis) or from adult tail and striatum dissected from adult mice brains (for somatic instability analysis) using the PureGene DNA isolation kit (Gentra, Minneapolis, MN, USA). Genotyping of the Hdh^{Q111} knock-in allele was carried out using a combination of a human-specific PCR assay that amplifies the *HD* CAG repeat from the knock-in allele but does not amplify the mouse sequence, and a wild-type mouse-specific assay that generates a PCR product from the wild-type mouse allele but not from the knock-in allele. The human-specific assay has been previously described (Mangiarini et al., 1997). For the wild-type mouse-specific assay primers 5′-CCTGGAAAAGCTGATGAAGG (forward) and 5′-TGGACAGGG AACAGTGTTGGC (reverse) were used in a PCR buffer containing 67 mM Tris–HCl pH 8.8, 16.7 mM (NH4)2SO4, 2 mM MgCl2, 0.17 mg/mg BSA, 10 mM 2-mercaptoethanol, 10% DMSO, 200 μM dNTPs, 5 ng/μl primers with 0.5 U *Taq* polymerase (Perkin Elmer). Cycling conditions were 94ϒC 90 s, 35 cycles of 94ϒC 30 s, 56ϒC 30 s, 72ϒC 90 s, followed by 10 min at 72ϒC. Products were resolved in 0.8% agarose gels. DNA repair knockout mice were genotyped as described (Cheo et al., 1997; Edelmann et al., 2000; Edelmann et al., 1997; Toft et al., 1999).

HD CAG repeat size was determined using the human *HD* CAG repeat-specific PCR assay (Mangiarini et al., 1997). The forward primer was fluorescently labeled with 6-FAM (Perkin Elmer) and products were resolved using either the ABI 377 or AB1 3730xl automated DNA analyzer (Applied Biosystems). GeneScan and Genotyper software packages with GeneScan 500-TAMRA as internal size standard (ABI 377) or GeneMapper v3.7 with GeneScan 500-LIZ as internal size standard (ABI 3730) were used to assign repeat size. Runs included the same control DNAs of known *HD* CAG repeat size. The *HD* CAG size was assigned as the highest peak in the GeneScan trace.

Analysis of intergenerational instability

For assessment of intergenerational instability, breeding pairs were established between 2 and 4 different $Hdh^{Q111/+}$ mice of each sex and DNA repair gene genotype (+/+, +/− and −/−) and $Hdh^{+/+}$ wild-type littermates that were either +/+ or +/− for the DNA repair gene mutation. Genomic DNA was extracted from tail biopsies of $Hdh^{Q111/+}$ transmitting parents and $Hdh^{Q111/+}$ progeny at weaning. Amplification of the *HD* CAG repeat and repeat size analysis were carried out as described above. Intergenerational instability was determined by comparing *HD* CAG repeat size in the $Hdh^{Q111/+}$ transmitting parent with those in the $Hdh^{Q111/+}$ progeny. Parent and pups were compared in the same ABI automated DNA sequencer run.

Immunohistochemistry

Immunohistochemistry was carried out on 7 μm paraffin-embedded coronal sections of brains perfused with periodate–lysine–paraformaldehyde as described (Wheeler et al., 2002). Immunostaining with polyclonal anti-huntingtin antibody EM48 (amino acids 1–256) (Gutekunst et al., 1999) was as described (Wheeler et al., 2002). All EM48 immunostaining experiments were performed under identical conditions. Diffuse EM48 immunostaining was quantified in striata from 3–5 $Hdh^{Q111/+}$ mice of each DNA repair genotype (+/+, +/−. −/−) to be investigated at 5 months of age. The mean nuclear stain intensity was determined in four 750 μm × 500 μm regions of dorsal striatum, matched in terms of their anterior/posterior location, using the 'histogram' function in Adobe Photoshop to convert the signal intensity in all stained nuclei to arbitrary units. Background signal, the mean value for 10 fields within each 750 μm × 500 μm region analyzed,