Huntington's disease

https://doi.org/10.1038/s41588-024-02055-4

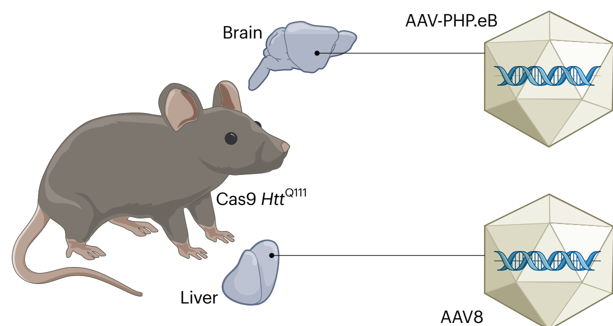
Defining genes and pathways that modify huntingtin CAG repeat somatic instability in vivo

Suphinya Sathitloetsakun & Myriam Heiman

A novel in vivo CRISPR screening platform identifies genetic modifiers of huntingtin CAG repeat somatic instability. These modifiers include known and novel genes that are promising therapeutic targets for Huntington's disease.

All cases of Huntington's disease are caused by CAG trinucleotide repeat expansions in exon 1 of the huntingtin (*HTT*) gene[1]. While the number of inherited CAG repeats in *HTT* predicts age of Huntington's disease onset, human genome-wide association studies (GWASs) have revealed that there are also genetic modifiers that can modulate age of onset[2,3]. Some of these GWAS-implicated genes encode known components of the DNA mismatch repair (MMR) pathway, which in mammals is carried out by homologs of MutS and MutL, and several of these GWAS genes have been previously shown to modify CAG repeat expansion somatically (*Msh2*, *Msh3*, *Mlh1* and *Mlh3* enhancing expansion and *Fan1* suppressing expansion) in mouse models of Huntington's disease[4]. These DNA repair genes probably have their effects through faulty processing of CAG/CTG DNA repeat loop-outs that occur during transcription or chromatin opening and remodeling of the *HTT* gene. GWASs provide compelling evidence for somatic CAG expansion being a crucial step leading to Huntington's disease pathogenesis, supported by the association of longer somatic *HTT* CAG repeat expansions in the brain with earlier age of Huntington's disease onset[5] and by the observation that the most affected cell types in Huntington's disease, striatal spiny projection neurons and deep layer cortical cells, harbor large somatic *HTT* CAG repeat expansions in human tissue[6,7]. However, it has not been clear whether all of the Huntington's disease age-of-onset modifier GWAS-implicated genes can modify somatic CAG expansion in vivo and in what cell types they might act if so. Furthermore, the interactions between these genes have not been clear, as most often gene effects in mouse models have been tested individually. To address these questions, in this issue Mouro Pinto et al.[8] devised an elegant adeno-associated virus (AAV) in vivo CRISPR–Cas9 screening platform (Fig. 1) to test the effects of GWAS-implicated Huntington's disease age-of-onset modifiers, as well as other genes that have been implicated in trinucleotide repeat instability in other contexts, on *Htt* CAG repeat somatic instability.

The study used CRISPR–Cas9-bearing Huntington's disease mice carrying an allele of the *Htt* gene with 112–119 CAG repeats (Htt^{Q111} knockin mice), a model that has been previously validated to reveal the genetic effects of CAG repeat modifiers[4]. AAV8- and AAV-PHP.eB-mediated guide RNA delivery were used to test for effects of gene inactivation on *Htt* CAG somatic instability in the liver or brain, respectively. In the liver, inactivation of *Pms1*, *Pold1* and *Pold3* suppressed CAG repeat expansion to a similar extent as inactivation of *Msh2*, *Msh3*, *Mlh1* and *Mlh3* (members of the MMR pathway that were previously shown to enhance CAG expansion), whereas inactivation of *Pold2*, *Pold4*, *Pole*, *Polb*, *Crebbp*, *Ercc1*, *Ercc5*, *Ercc3*, *Setd2* and *Setdb1* also suppressed CAG repeat expansion, but to a lesser degree. Inactivation of *Pms2*, *Msh6*, *Hmgb1* and *Lig4* increased CAG repeat expansion, but to a lesser degree than the known effect of inactivating *Fan1*. Similar results were seen in the striatum, with the exception that the weak modifying effects of *Msh6* inactivation were not seen in the striatum, and *Pms2* inactivation had an effect of higher magnitude in the striatum than in the liver, both findings pointing toward the likely existence of tissue-specific effects



Candidate genes

- Huntington's disease onset modifier GWAS genes
- DNA repair/metabolic genes
- Other repeat instability-associated genes

In vivo CAG instability supressors	In vivo CAG instability enhancers
Fan1	*Msh2, Msh3, Mlh3*
Pms2	*Mlh1, Pms1, Pold1, Pold3*
Hmgb1	*Pold2*
Lig4	*Pold4*
Msh6	*Pole, Polb, Crebbp, Ercc1, Ercc5*
	Ercc3, Setd2, Setdb1

Fig. 1 | In vivo CRISPR screening identifies *HTT* CAG instability modifiers. The liver and striatum of Htt^{Q111} knockin mice were targeted in this AAV-based screen that assessed effects of genes drawn from various sources of implication (candidate genes). Effects were seen across genes belonging to various biological pathways. Bottom right: blue color is used to denote genes that suppress CAG somatic instability and red color is used to denote genes that enhance CAG somatic instability; deeper color denotes stronger relative magnitude of effect.

of these modifiers. The authors then used the liver screening system to test for interactions between candidate modifier genes, finding that the effects of CAG instability suppressors *Fan1*, *Pms2* and *Msh6* are dependent on the CAG instability enhancers *Msh2*, *Msh3*, *Mlh1*, *Pms1* and *Mlh3*, while *Msh6* inactivation reduced the effects of *Fan1* and *Pms2* inactivation.

This study validates several in vivo findings from other systems, but also identifies differences, highlighting the importance of delineating genetic modifiers in vivo and in disease-relevant tissues. It reveals that the MMR pathway complexes MutLβ (MLH1–PMS1) and MutLγ (MLH1–MLH3), as well as DNA polymerase δ (Polδ, involved in gap-filing synthesis in MMR) and to a lesser extent Polβ and Polε, contribute to *Htt* CAG expansions in vivo, whereas the MutLα (MLH1–PMS2) complex suppresses *Htt* CAG repeat expansions in vivo. It is possible that these results are in some cases dependent on gene context, cell-type context or expression level, as for example *Pms2* has been linked to different effects on repeat expansions, including in a recent in vitro CRISPR interference study testing the effects of MutS, MutL and *LIG1* on *HTT* CAG instability in a human ex vivo system[9]. The results of this study also help to clarify that the effects of Huntington's disease GWAS age-of-onset modifier genes *TCERG1* and *CCDC82* are probably through other contributions that do not involve modulating CAG repeat instability. The effects of *Lig4* inactivation (previously linked to CGG repeat instability[10]) suggest that other members of the double-strand break repair pathway should be tested for effects on CAG repeat instability in future studies. Further, the effects of *Ercc1*, *Ercc3*, *Ercc5*, *Crebbp*, *Setd2*, *Setdb1* and *Hmbg1* all support the notion that has emerged from recent studies (for example, ref. 11) that transcription through, or else chromatin opening of, the CAG repeat region is necessary for CAG repeat instability. The study ends by proposing a model whereby the MutSβ (MSH2–MSH3) and MutLγ (MLH1–MLH3) complexes have primary roles in regulating CAG repeat expansion that are either facilitated or inhibited by other modifying factors. Future studies with this versatile platform for genetic testing, for example genome-wide testing with single-cell readouts, hold promise to reveal many further insights into potential Huntington's disease therapeutic targets that halt *HTT* CAG somatic expansion.

Suphinya Sathitloetsakun [1,2] **& Myriam Heiman**[1,3] ✉

[1]The Picower Institute for Learning and Memory, Cambridge, MA, USA. [2]Department of Biology, MIT, Cambridge, MA, USA. [3]Department of Brain and Cognitive Sciences, MIT, Cambridge, MA, USA.

✉e-mail: mheiman@mit.edu

Published online: 22 January 2025

References

1. The Huntington's Disease Collaborative Research Group. *Cell* **72**, 971–983 (1993).
2. Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium. *Cell* **162**, 516–526 (2015).
3. Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium. *Cell* **178**, 887–900.e14 (2019).
4. Wheeler, V. C. & Dion, V. *J. Huntingtons Dis.* **10**, 123–148 (2021).
5. Swami, M. et al. *Hum. Mol. Genet.* **18**, 3039–3047 (2009).
6. Mätlik, K. et al. *Nat. Genet.* **56**, 383–394 (2024).
7. Pressl, C. et al. *Neuron* **112**, 924–941.e10 (2024).
8. Mouro Pinto, R. et al. *Nat. Genet.* https://doi.org/10.1038/s41588-024-02054-5 (2025).
9. Ferguson, R., Goold, R., Coupland, L., Flower, M. & Tabrizi, S. J. *Am. J. Hum. Genet.* **111**, 1165–1183 (2024).
10. Gazy, I., Hayward, B., Potapova, S., Zhao, X. & Usdin, K. *DNA Repair* **74**, 63–69 (2019).
11. McLean, Z. L. et al. *Nat. Commun.* **15**, 3182 (2024).

Competing interests

The authors declare no competing interests.