FAN1 controls CAG repeat expansion in Huntington's disease by dual functions, MLH1 retention and nuclease activity

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Background

Somatic expansion in Huntington's disease is a pathogenic phenomenon whereby the CAG trinucleotide repeat in exon 1 of the *HTT* gene expands throughout an individual's lifetime. This occurs via the mismatch repair (MMR) pathway, requiring key proteins MSH3 and MLH1, involved in excising DNA loop-outs.

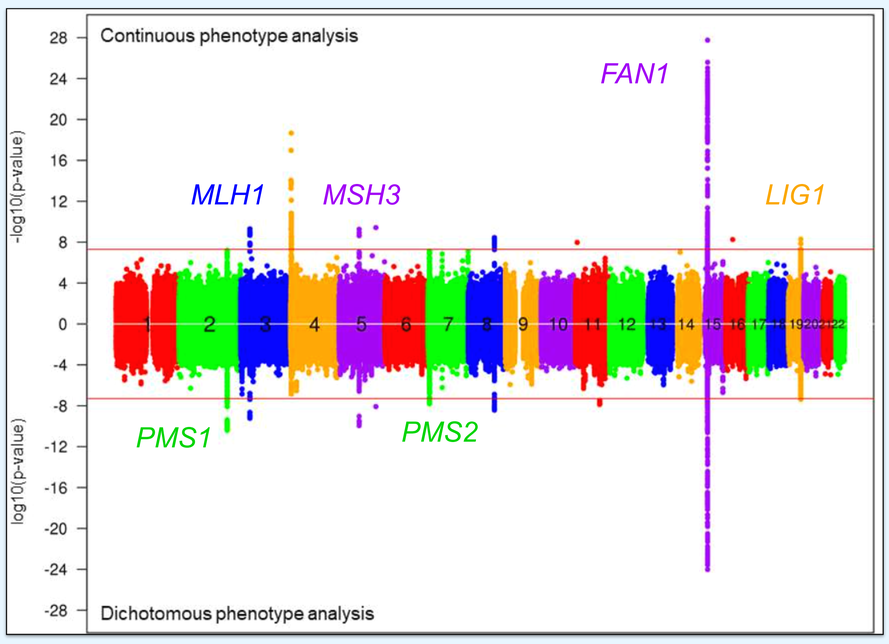

Figure 1. *Manhattan plot showing individual SNPs in DNA repair genes identified as modifiers of HD age at onset (adapted from GeM-HD Consortium, 2019)*

Recent GWAS evidence highlighted the DNA repair gene *FAN1* as a HD-modifier (1), which acts by suppressing somatic expansion (2). This project establishes the mechanism by which FAN1 stabilises the *HTT* CAG repeat.

Results (I)

FAN1 binds MLH1 via residues ^{126}SPYF^{129}, reducing somatic expansion

A genetic analysis identified an evolutionary conserved motif, ^{126}SPYF^{129}, resembling an MLH1-interacting protein (MIP)-box found in known MLH1-interactors such as MSH3. Mutation of these residues essentially abolished the FAN1-MLH1 interaction and reduced somatic expansion (**Fig. 3**)

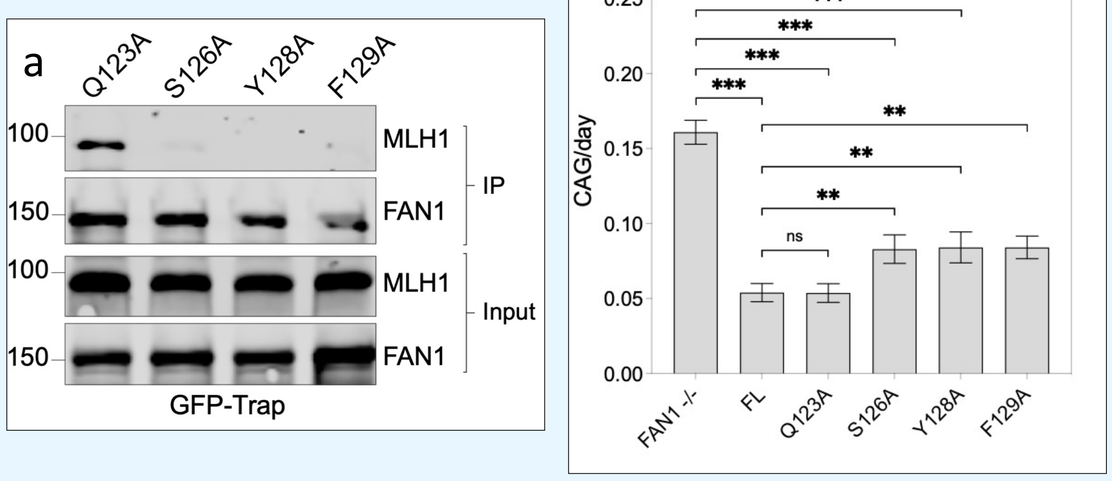

Figure 3. (a) *GFP-Trap pulldown of FAN1 in U2OS cells showed reduced MLH1-binding after mutation of ^{126}SPYF^{129} residues.* ***(b)*** *Mutation of ^{126}SPYF^{129} residues reduced somatic expansion significantly, although not as pronounced as FAN1$^{-/-}$ cells.*

FAN1's nuclease domain accounts for residual stability mechanism

FAN1's nuclease domain was inactivated through the D960A mutation. In concert with mutation of the MIP-box (F129A), this produced a somatic expansion rate equivalent to FAN1-/- cells (**Fig. 4**)

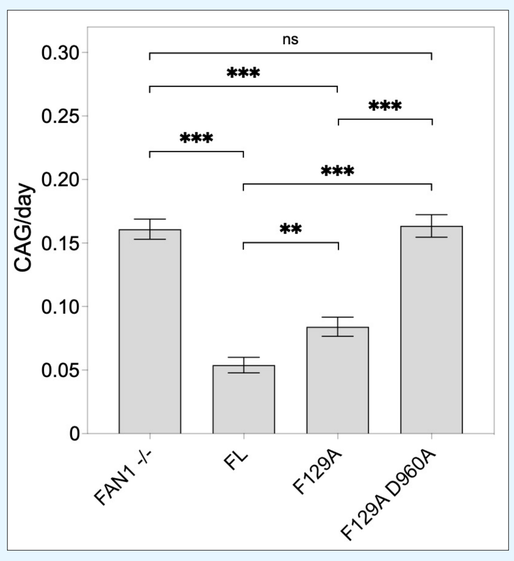

Figure 4. *Somatic expansion rates in U2OS cells expressing FAN1 variants with MLH1-binding mutation F129A and nuclease-dead mutation D960A.*

Methods

The FAN1$^{-/-}$ U2OS osteosarcoma model allows the effects of FAN1 variants on somatic expansion and DNA repair protein interactions to be assessed (**Fig. 2**).

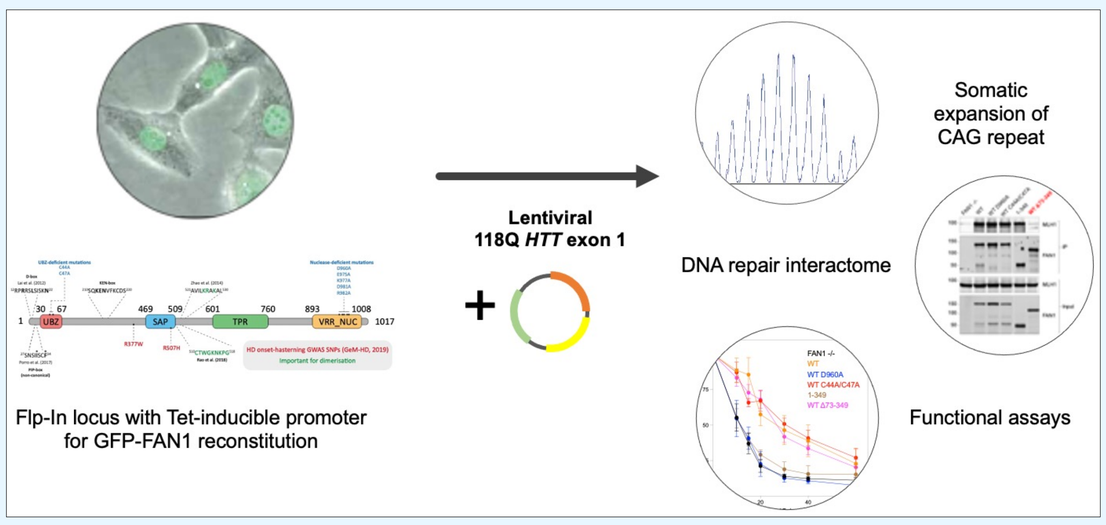

Figure 2. *FAN1-/- U2OS cell model featuring a Flp-In locus for complementation with GFP-tagged FAN1 variants. Cells are co-transduced with a 118Q HTT exon 1 construct.*

Somatic expansion was measured by fragment analysis: CAG repeat tracts were PCR-amplified with fluorescent forward primers and resolved by capillary electrophoresis.

Results (II)

FAN1-MLH1 binding reduces somatic expansion by limiting mismatch repair

FAN1 knockdown in 125 CAG medium spiny neurons (MSNs) promoted a greater MSH3-MLH1 interaction, known to drive somatic expansion through MMR activity (**Fig. 5a**). Concurrently, MIP-box *FAN1* mutants showed reduced viability in a 6-thioguanine assay, indicative of increased mismatch repair (**Fig. 5b**).

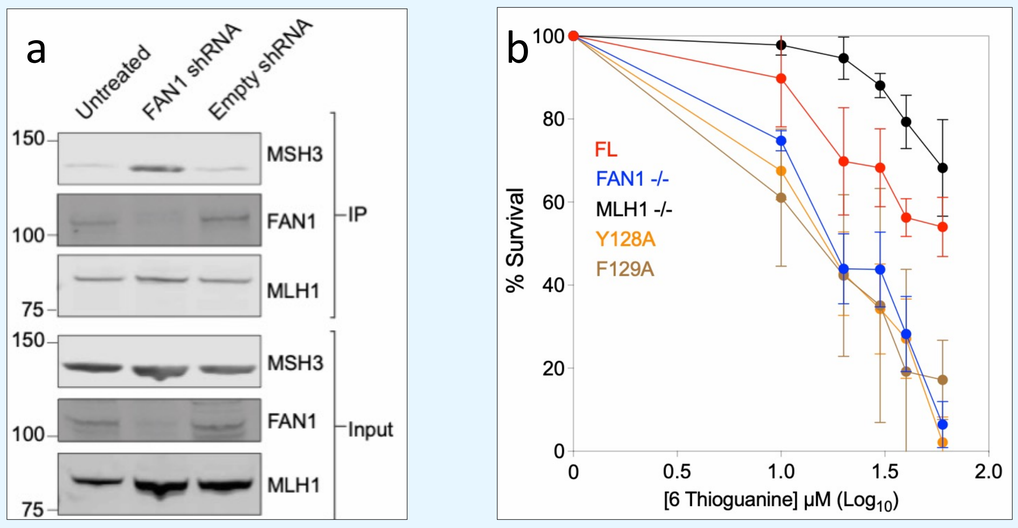

Figure 5. (a) *MLH1 IP in 125 CAG MSNs showed greater MSH3-MLH1 binding in the absence of FAN1.* ***(b)*** *Mutation of ^{126}SPYF^{129} residues in U2OS cells reduced viability in 6-thioguanine assay, suggestive of greater MMR activity.*

Conclusions

FAN1 stabilizes the *HTT* CAG repeat by MLH1 sequestration from the MMR pathway and nuclease activity (**Fig. 6**). Promoting this interaction may alter pathology and the impact of doing so will be subsequently examined in physiologically-relevant HD systems.

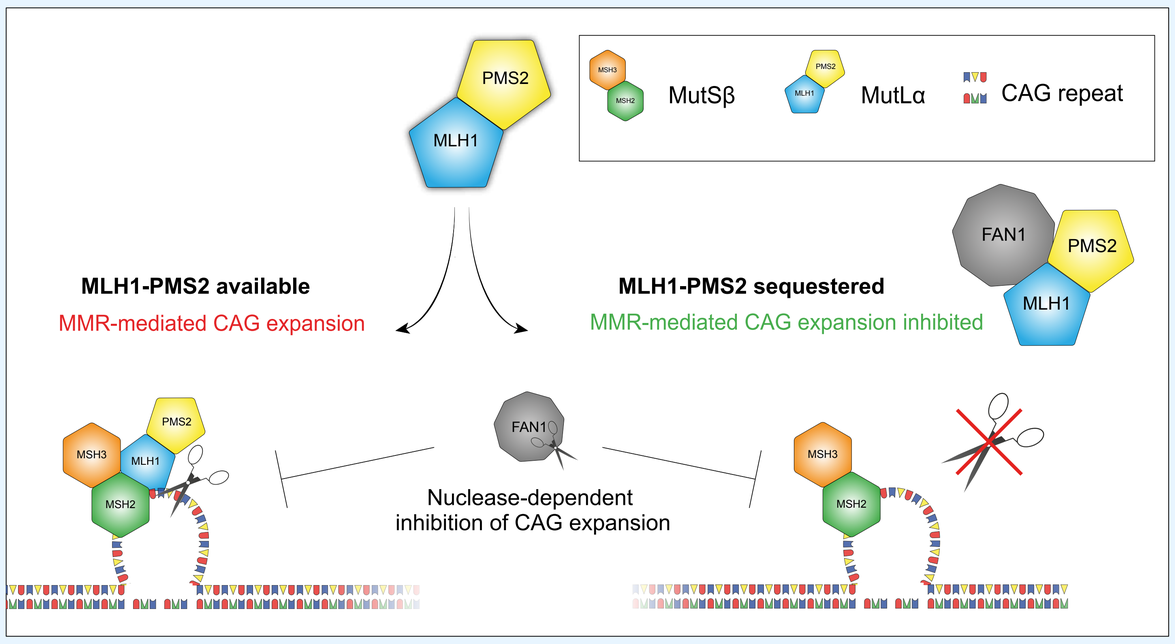

Figure 6. *Proposed mechanism of FAN1 in limiting somatic expansion at the CAG repeat*

References

1. GeM-HD Consortium (2019) CAG repeat not polyglutamine length determines timing of Huntington's disease onset. Cell, 178, 887-900 e14.
2. Goold R, Flower M, Hensman-Moss D, Medway C, Wood-Kaczmar A, Andre R, Farshim P, Bates GP, Holmans P, Jones L, Tabrizi SJ (2018) FAN1 modifies Huntington's disease progression by stabilising the expanded HTT CAG repeat. *Hum Mol Genet* 0: 1-12