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Pcaf Modulates Polyglutamine Pathology in a *Drosophila* Model of Huntington's Disease

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Polyglutamine · Huntington's disease · Histone acetyltransferase · Pcaf · Nejire · *Drosophila*

Abstract
Huntingtin peptides with elongated polyglutamine domains, the root causes of Huntington's disease, hinder histone acetylation, which leads to transcriptional dysregulation. However, the range of acetyltransferases interacting with mutant Huntingtin has not been systematically evaluated. We used genetic interaction tests in *Drosophila* to determine whether specific acetyltransferases belonging to distinct protein families influence polyglutamine pathology. We found that flies expressing a mutant form of the Huntingtin protein (Httex1pQ93) exhibit reduced viability, which is further decreased by partial loss of *Pcaf* or *nejire,* while the tested MYST family acetyltransferases did not affect pathology. Reduced levels of *Pcaf* also led to the increased degeneration of photoreceptor neurons in the retina. Overexpression of *Pcaf,* however, was not sufficient to ameliorate these phenotypes, and the level of soluble Pcaf is unchanged in Httex1pQ93-expressing flies. Thus, our results indicate that while Pcaf has a significant impact on Huntington's disease pathology, therapeutic strategies aimed at elevating its levels are likely to be ineffective in ameliorating Huntington's disease pathology; however, strategies that aim to increase the specific activity of Pcaf remain to be tested.

Huntington's disease (HD) belongs to a group of neurodegenerative diseases caused by the expansion of a polyglutamine (polyQ) domain in the affected protein. Transcriptional dysregulation is one of the molecular mechanisms underlying polyQ pathology [1]. The effect on transcription can be partially explained by the fact that mutant Huntingtin (Htt) binds to several transcription factors [1]. One group of affected factors is the histone acetyltransferases (HATs) [2]. Mutant Htt binds to CBP, p300 and Pcaf and inhibits their activities leading to a decreased level of histone acetylation [3]. Furthermore, mutant Htt depletes CBP by targeting it for degradation [4], and depletion of CBP is associated with cell death in HD models [5]. The importance of altered protein acetylation in HD is underscored by experiments showing that inhibition of histone deacetylase enzymes ameliorates HD phenotypes in several animal models [3, 6, 7]. Although the HATs belong to different protein families, for example the GNAT (e.g. Pcaf), the MYST and the CBP/p300 families [8], most studies to date have focused only on the role of CBP in disease pathogenesis leaving the role of other HATs unknown. Here we describe a genetic interaction study designed to determine whether HATs other than CBP are involved in polyQ pathogenesis.

To investigate the consequences of reduced HAT activity on polyQ pathology, we used a *Drosophila* model of HD, which was previously shown to be sensitive to acetylation levels [3, 6]. We compared the phenotypes of flies that express Httex1pQ93 in the nervous system with their

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Table 1. Results of genetic interaction crosses involving HAT mutants and Htt

HAT family	Affected gene	Mutation	Relative eclosion, %	p value	n	HAT mutation; Htt exp.	Htt exp.	HAT mutation; no Htt	No Htt
CBP/p300	*nej*	nej^{3}	67.3	<0.01	1,268	150	209	469	440
MYST	*enok*	$enok^{2}$	92.5	0.47	2,341	202	208	989	942
	mof	mof^{2}	100.5	0.96	1,437	232	206	528	471
	CG1894	*Df(3R)Ex6259*	83.7	0.12	1,432	197	215	533	487
GNAT	*Pcaf*	$Pcaf^{E333St}$	49.4	<0.001	3,419	114	215	1,600	1,490
		Df(3L)iro-2	54	<0.001	4,163	340	612	1,629	1,582
		$UAS\text{-}Pcaf^{1}$	102.2	0.89	1,002	99	88	427	388
		$UAS\text{-}Pcaf^{3B}$	118.4	0.3	785	109	93	290	293
		$UAS\text{-}Pcaf^{5L}$	135.5	0.08	867	89	74	331	373
		$UAS\text{-}Pcaf^{7}$	126.2	0.32	390	51	46	137	156

The relative eclosion rates, statistical significance, number of all scored progenies (n) and the number of offspring in the four genotype categories are shown for loss of function HAT mutants, HAT deletions and UAS-Pcaf overexpressor lines.

siblings that, in addition, carry a HAT mutation as well. We found that partial loss of *Pcaf* (the single fly homolog of human Pcaf and Gcn5) or *nejire/dCBP* (*nej,* homolog of human CBP) by the $Pcaf^{E333St}$ or nej^{3} mutations, respectively, significantly reduced the viability of *Htt*-expressing flies, while reducing the MYST family acetyltransferases *enok*, *mof* or *CG1894* did not have a significant effect on pathology (table 1).

Since the role of CBP is well established in HD pathogenesis, we sought to investigate in detail the effect of *Pcaf,* which was not previously characterized. First, we tested whether *Df(3L)iro-2,* an independent deletion that removes the *Pcaf* gene but does not share the same genetic background as the $Pcaf^{E333St}$ null allele, has the same effect. We found that Htt-expressing flies heterozygous for *Df(3L)iro-2* also exhibited significantly reduced viability by 46% (table 1). Next we asked whether reduced Pcaf levels lead to neuronal toxicity. We compared *Htt*-expressing control flies with siblings expressing *Htt* and also carrying the $Pcaf^{E333St}$ allele, and found that the average number of rhabdomeres (light gathering structures of photoreceptor neurons) per ommatidium decreased from 4.66 ± 0.08 to 4.32 ± 0.11 (n = 10, p = 0.021), as measured by the pseudopupil assay. We obtained a similar result with the *Df(3L)iro-2* deletion, where the average number of rhabdomeres decreased from 4.78 ± 0.11 to 4.42 ± 0.03 (n = 7, p = 0.028), indicating that reduced Pcaf levels enhance neurodegeneration.

Since reducing *Pcaf* is deleterious, we next asked whether Htt pathology could be ameliorated by overexpressing *Pcaf*. We generated transgenic flies expressing a full-length *Pcaf* cDNA under UAS control and tested four independent *UAS-Pcaf* transgenic strains. We found that the eclosion rates of flies expressing both *Pcaf* and *Htt* simultaneously in the nervous system were slightly, albeit not significantly, higher than those of mutant *Htt*-expressing control siblings (table 1). Similarly, we found no significant difference in neurodegeneration between flies coexpressing *Htt* and *Pcaf,* compared to siblings expressing only *Htt,* as measured by the pseudopupil assay (data not shown).

In addition, we sought to determine whether the quantity of soluble Pcaf protein is altered in *Httex1pQ93*-expressing flies and found that mutant Htt expression does not result in the reduction of the level of Pcaf (fig. 1).

Taken together, we found that partial loss of *Pcaf* significantly enhanced polyglutamine pathology, but the level of Pcaf was not reduced by mutant Htt, and we could not rescue HD phenotypes by the overexpression of *Pcaf,* indicating that the interaction of mutant Htt and Pcaf does not involve the depletion of soluble Pcaf by either degradation or sequestration to insoluble aggregates. This result, however, does not exclude the possibility that a soluble toxic form of Htt might inhibit the function of either Pcaf itself or of Pcaf-containing complexes. Since Pcaf acts as a catalytic subunit in large multiprotein complexes in metazoans [9], we speculate that its interaction with a polyQ peptide might cripple entire complexes, which cannot be rescued by overexpression of *Pcaf* alone. We conclude that although *Pcaf* has a significant impact on HD pathology, therapeutic strategies aimed at elevating the levels of Pcaf protein are unlikely to be effective in ameliorating HD pathology. The question, however,

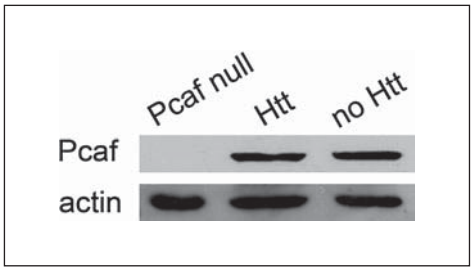

Fig. 1. Mutant Huntingtin does not reduce the level of soluble Pcaf. Immunoblots of head extracts of Httex1pQ93-expressing flies (Htt) are shown next to wild-type control (no Htt), as well as larval extracts of homozygous $Pcaf^{E333St}$ (Pcaf null).

remains open whether strategies that aim to increase the specific activity of Pcaf might be useful. Interestingly, of the three HAT families of proteins tested, only the GNAT and the CBP/p300 families exhibit a strong influence over HD pathology, while the MYST family members have decidedly less impact.

Materials and Methods

Stocks carrying the mutations $Pcaf^{E333St}$, *Df(3L)iro-2, Df(3R) Exel6259*, $enok^{2}$, nej^{3}, and the pan-neuronal elav-GAL4 driver *w P{w*$^{+mW.hs}$*=GawB}elav*^{C155} were from the Bloomington Drosophila Stock Center. The mof^{2} allele was kindly provided by John C. Lucchesi (Emory University, Atlanta, Ga., USA). The *w; P{UAS-Httex1p-Q93}4F1* transgenic line expressing the first exon of human Htt with a 93-residue-long polyQ repeat under UAS control was generated in our laboratory previously [3].

UAS-Pcaf transgenic lines were produced by cloning the full-length *Pcaf* cDNA from the GH11602 clone (from the Drosophila Genomics Resource Center, Bloomington, Indiana University) to the EcoRI site of pUAST, and generating transgenics by standard P element-mediated transformation.

Viability tests were done by crossing *elav>Gal4/Y HAT mutant/Marker* males to *Httex1pQ93/Httex1pQ93* females and scoring the number of offspring in the four genotype categories. Relative eclosion rates were calculated as the ratio of HAT mutant to HAT wild-type *Htt*-expressing siblings normalized by the ratio of HAT mutant to HAT wild-type siblings not expressing *Htt*. The statistical significance of differences in eclosion was determined by a χ^2 probe. Pseudopupil analysis was performed on 6-day-old females as described previously [3]; statistical significance was evaluated by unpaired t test.

Immunoblotting was performed on head extracts from 1-day-old flies using polyclonal antibodies against Pcaf/dGcn5 [10] and actin (Sigma A5060) with goat-anti-rabbit-HRP secondary antibody (Dako P0448); detection was done using Immobilon Western Chemiluminescent HRP substrate (Millipore).

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