**PROJECT CLOSING REPORT**

**Specific histone modifications in the pathogenesis of Huntington's disease (grant no. K112294)**

## Generation and characterization of genetic tools used in the study

The main goal of the project was to elucidate the role of specific chromatin marks in the pathogenesis of Huntington's disease (HD). We used a *Drosophila* model of HD in our experiments, which is based on the neuronal expression of mutant Huntingtin (Htt). To be able to perform genetic interaction tests providing information about the effects of specific histone modifications on mutant Htt induced pathogenesis we generated transgenic flies using transgenes of histone variants with missense mutations mimicking posttranslational modifications (PTM) of histones. We cloned Drosophila *His3.3A* and *His4r* and used site directed mutagenesis to introduce mutations resulting in lysine (K) – arginine (R) change to mimic non-modified-K; lysine – glutamine (Q) to mimic acetyl-K; and lysine-methionine (M) to mimic methyl-K at specific residues. We subcloned the modified histone genes to pTWF-attB vector, which enables site directed integration, GAL4 dependent transcription and C-terminal FLAG-tagging, and generated transgenic flies by site specific transformation. Using this method we generated 19 transgenic strains: UAS-His3.3A, UAS-His3.3A-K9R, UAS-His3.3A-K9Q, UAS-His3.3A-K9M, UAS-His3.3A-K14R, UAS-His3.3A-K14Q, UAS-His3.3A-K27R, UAS-His3.3A-K27Q, UAS-His3.3A-K27M, UAS-His4r, UAS-His4r-K5R, UAS-His4r-K5Q, UAS-His4r-K8R, UAS-His4r-K8Q, UAS-His4r-K12R, UAS-His4r-K12Q, UAS-His4r-K16R, UAS-His4r-K16Q and UAS-His4r-K5R-K12R.

We validated the transgenic strains at several levels. We validated the mutations by sequencing, confirmed transgene expression by RT-qPCR and immunoblot analysis, and confirmed that all mutant derivatives of both H3.3A and His4r localized correctly to the nucleus by immunhistochemistry.

We analyzed the effects of neuronal expression of histone transgenes on viability (eclosion) using the pan-neuronal elav-GAL4 driver. Neuronal expression of His4r transgenes did not have a significant effect on viability. In case of H3.3A derivatives, PTM mimics affecting K27 residue (K27M and K27Q) significantly reduced viability, while the other transgenes did not affect it.

We obtained transgenes expressing the first exon of human Htt with elongated (Httex1Q120) or normal length (Httex1Q25) polyglutamine repeats. We showed that neuronal expression of Httex1Q120 causes reduced viability, reduced lifespan, neurodegeneration and reduced climbing abilities.

We studied the expression pattern of Appl-GAL4, a potential alternative of elav-GAL4 for neuronal expression. We found that Appl-GAL4 does not provide uniform expression in adult

brains, its expression level is focused on the mushroom body and on the ventral nerve cord. [*Kókity L & Bodai L. Appl-GAL4 driven transcription in adult heads. Dros. Inf. Serv. 2017. 100:54*] Expression of a mutant Htt by Appl-GAL4 showed lower toxicity than if expressed by elav-GAL4.

To be able to investigate the effects of Htt and mutant histone variant transgenes expressed in the adult nervous system in an inducible manner we obtained and utilized GAL80[ts] lines that express a heat sensitive allele of GAL80, a repressor of GAL4. We published the molecular characterization of the transgenes in [*Siági F, Zsindely N, Bodai L. Genomic localization of two public gal80ts transgenes. Dros. Inf. Serv. 2015. 98:98.*].

We also obtained null mutants of histone variants (*Df(2L)His3.3A* and *Df(X)H3.3B*), and generated all genetic combinations of GAL4 drivers, Htt transgenes, GAL80[ts] elements, histone PTM transgenes and histone variant mutants that were required to be able to perform the planned genetic interaction tests.

### Circadian rhythm and sleep patterns are disturbed in HD flies

To be able to characterize the effects of mutant Htt expression on the function of the neuro-muscular system (beside the well-established climbing assays) we obtained TriKinetics activity monitors designed to continuous movement activity recording. Using these monitors we observed that although mutant Htt expressing flies perform worse (are slower) in climbing assays, they are hyperactive, move more than controls. We performed movement circadian rhythm analysis and found that hyperactivity is due to disturbed sleep patterns: Htt flies sleep less, the average length of sleep episodes is shorter, the number of sleep episodes is higher during daytime, and the time required to get asleep is longer. We also showed that the temporal transcriptional pattern of circadian rhythm control genes *period*, *timeless*, *Clock* and *vrille* was altered in HD flies. We investigated whether histone acetylation has a role in disturbed sleep patterns in HD flies and found that overexpression of the histone acetyltransferase CBP corrected sleep patterns. [*manuscript submitted to Scientific Reports (currently under minor revision): Faragó A, Zsindely N, Bodai L. Mutant huntingtin disturbs sleep patterns and circadian clock gene expression in Drosophila.*]

### Testing the effects of His3.3A mutants on HD pathology

We investigated the potential role of three important post-translation modification sites of histone H3.3A in HD pathogenesis: K9, K14 and K27. We performed the experiments on a *Df(2L)His3.3A* hetero- or homozygous background to lower His3.3A load, which was elevated by transgene expression. We studied several Htt induced phenotypes. First, we performed experiments with elav-GAL4 that provides neuronal expression and analyzed the effects on reduced viability (eclosion rate), reduced lifespan and neurodegeneration in the eye

(pseudopupil assay). Next, we used elav-GAL4 along with tub-GAL80[ts] that allowed inducible, adult only neuronal expression, and analyzed reduced lifespan, motor capabilities (climbing assay) and daily motor activity.

In these experiments both transgenes (UAS-Htt and UAS-H3.3A) were driven by the same GAL4 source, therefore we investigated whether the level of HttQ120 is decreased by the presence of the UAS-H3.3A constructs. We performed RT-qPCR measurements and found no difference in the level of HttQ120 transcripts proving that the observed phenotypical changes are not due to decreased Htt levels.

Before testing PTM mimic mutants we analyzed whether reduction or overexpression of H3.3A influences HD phenotypes. We found that heterozygous Df(2L)His3.3A increased longevity of Httex1Q120 expressing flies. Next, we studied the effects of His3.3A overexpression on Httex1Q120 induced toxicity in flies that expressed transgenes throughout life and found that co-expression of UAS-His3.3A with UAS-Httex1Q120 had an enhancing effect on mutant Htt induced phenotypes.

**Acetylation mimic of Lysine 14 of histone H3 ameliorates Htt pathology**

We started our tests with H3.3A mutations affecting the K9 and K14 residues. By testing of H3.3A-K9 mutants we found that K9Q and K9R did not affect viability, but K9M significantly reduced viability. None of the mutant K9 transgenes affected reduced lifespan. Htt induced neurodegeneration was significantly enhanced by K9R but not affected by K9Q or K9M.

Next, we tested H3.3A-K14 mutants and found that K14Q significantly ameliorated Htt induced reduced viability and lifespan and neurodegeneration. On the other hand, K14R strongly enhanced Htt induced toxicity; because of the lack of survivors lifespan and neurodegeneration couldn’t be tested.

To avoid developmental defects due to through life transgene expression in the next set of experiments we used a combination of elav-GAL4 and tub-GAL8[ts] and drove transgene expression in adult neurons only.

Using this system, we found that K9M mutation increased lifespan, while K9Q and K9R significantly reduced it. None of these mutations, however, influenced motor capabilities in climbing assays, daily motor activity or the daily amount of sleep.

In case of K14 mutants, we found that K14Q had a positive effect in all tested phenotypes: it increased lifespan, increased motor performance in climbing assays, reduced motor hyperactivity and increased the daily amount of sleep. K14R had opposite effects: reduced lifespan and climbing performance, increased hyperactivity and reduced amount of sleep.

Next, we started experiments to investigate whether the H3.3A-K14R and H3.3A-K14Q mutations are epistatic over histone deacetylase (HDAC) inhibitors, SAHA and Na-butyrate, that were shown previously to have a positive impact on HD pathology. Our hypothesis was,

that if HDAC inhibitors act by modulating the acetylation level of the K14 residue then they will be ineffective in K14 PTM mimic mutants. We found that neither SAHA nor Na-butyrate could provide additional suppression of HD phenotypes of Httex1Q120 expressing flies in the presence of K14Q or K14R. Thus, these finding supported the hypothesis.

To determine whether H3K14 PTM mimic mutations affect transcriptomic changes in the HD model we performed RNA-seq analysis of flies expressing the following transgenes under the control of elav-GAL4 tub-GAL80[ts]: HttQ120, HttQ120 - H3.3A, Httex1Q120 – H3.3A-K14Q, Httex1 – H3.3A-K14R; and non-expressing controls. We found that in HttQ120 expressing flies 527 genes showed significantly altered transcript levels compared to controls. Significantly enriched Gene Onthology (GO) Biological Process categories defined by these genes were: regulation of organelle organization, negative regulation of gene expression, positive regulation of RNA metabolic process, regulation of transcription by RNA polymerase II, etc. Surprisingly, we found that additional expression of H3.3A transgenes beside Httex1Q120 caused minimal transcriptional changes: 14 genes showed differential expression between Httex1Q120 and HttQ120 - H3.3A; 20 genes showed differential expression between Httex1Q120 and HttQ120 - H3.3A-K14Q; and 31 genes showed differential expression between Httex1Q120 and HttQ120 - H3.3A-K14R. This suggests that the observed phenotypic changes are not due to correction or compensation of the transcriptional dysregulation present in HD; and the observed effects might be caused by other chromatin related mechanisms. [*manuscript under preparation*]

**H3K27 methylation state influences HD pathology**

In the following experiments we tested the effects of PTM mimic mutants affecting the H3.3A-K27 residue. Using the elav-GAL4 driver we found that K27R and K27M mutants enhanced Htt toxicity very strongly, they reduced viability to zero. Although K27Q mutation had milder effects, it still enhanced reduced viability, lifespan and neurodegeneration.

Next we tested the transgenes with adult neuronal expression using elav-GAL4; tub-GAL80[ts]. In this case K27Q and K27R significantly increased longevity, while K27M did not have an effect on lifespan. Furthermore, K27Q also increased performance in climbing assays, while K27R and K27M did not have an effect. None of the K27 mutants influenced daily activity or sleep.

As detailed above, we found that through life expression of K27 PTM mimics have strong developmental effects, but adult only expression of K27Q significantly increased longevity and climbing performance of HD flies. We further explored the role of H3K27 methylation in genetic interaction tests with methyltransferase and demethylase enzymes, and found that reduced activity of the K27 specific demethylase UTX by either genetic or pharmacological means (using GSK-J4 inhibitor) rescued Htt induced neurodegeneration; while reduced activity

of subunits of the K27 specific methyltransferase complex PRC2 enhanced pathology. However, we did not find pre-existing H3K27 methylation differences between HD flies and controls. [*Song W, Zsindely N, Faragó A, Marsh JL, Bodai L. Systematic genetic interaction studies identify histone demethylase Utx as potential target for ameliorating Huntington's disease. Hum Mol Genet. 2018. 27:649.*]

We also found the beside the state of the repressive methylation mark H3K27me3, the state of an activating methylation mark, H3K4me3 also influences HD pathology. From these we speculated that bivalent chromatin that simultaneously contain active and repressive epigenetic marks might have a role in HD pathogenesis. [*Zsindely N, Bodai L. Histone methylation in Huntington's disease: are bivalent promoters the critical targets? Neural Regen Res. 2018. 13:1191.*]

**Testing the effects of His4r mutants on HD pathology**

Before testing the effects of His4r PTM mimic mutants in HD pathology we aimed to determine whether reduced His4r levels affect mutant Htt induced phenotypes. For this, we generated a null mutant of His4r. His4r[Δ42] has a 695 bp deletion that removes the whole coding region of His4r. His4r[Δ42] homozygous flies are viable and fertile indicating that His4r is not an essential gene. We showed that neuronal expression of His4r drops to 50% by week 6, and to 20% by week 8 of adult life, however, His4r does not affect longevity. To test the effect of loss of His4r on chromatin organization we tested the effect of His4r[Δ42] on position effect variegation (PEV) and found that the mutation is a weak dominant suppressor of PEV. However, this effect is most likely does not influence gene expression as transcriptomic analysis showed only minor alteration in His4r mutants. We tested whether reducing the dose of His4r affects HD phenotypes and found that His4r[Δ42] deletion slightly increased the viability of flies expressing mutant Htt in the nervous system. [*manuscript under preparation*]

Next, we aimed to test the effects of His4r point mutations on elav-GAL4 driven mutant Httex1Q120 induced phenotypes. We investigated the potential role of four PTM sites of histone His4r (K5, K8, K12 and K16) in HD pathogenesis, using acetylation mimic K->Q, and unmodifiable K->R mutants. Before starting genetic interaction tests with Httex1Q120 we tested whether elav-GAL4 driven expression of His4r PTM mimic mutants alone affects phenotypes that we measure in our analysis, and found that there was no change in the viability and motor activity of any of the mutants and they did not show neurodegeneration. Thus, we started testing genetic interactions between Httex1pQ120 and His4r PTM mutants. We found that K8Q and K16Q increased viability, while K5R, K8R, K12R and K12Q reduced it compared to control. K8Q and K16Q also increased longevity compared to control, while other transgenes had no or negative effect. We also found that K5Q, K16Q and K16R ameliorated Htt induced neurodegeneration, while other transgenes did not affect neurodegeneration.

We also tested the effects of the transgenes in adult neurons only using elav-GAL4; tub-GAL80[ts]. In longevity assays, none of the PTM mimic transgenes increased lifespan compared to controls. None of the transgenes had significant positive impact on motor capabilities of HD flies in climbing assays, K5R transgenes had a significant negative impact on the phenotype.

In conclusion, interaction tests with H4 PTM mimic transgenes did not provide a clear picture yet, we treat K8Q and K16Q as potential candidates.

**Loss of Hat1 cytoplasmic acetyltransferase affects transcription and suppresses Htt induced neurodegeneration**

Analysis of HAT genes in HD was limited to type-A, nuclear HATs, while the potential effects of B-type, cytosolic HATs, that act in the cytosol and might influence histone incorporation and exchange, were not tested before. To be able to analyze the potential effect of cytosolic HATs in HD, we generated mutants of the putative B-type HAT gene, Hat1. We generated a deletion mutant, Hat1[Δ57], that removes 1419 bp (80% of the coding region) of Hat1. We found that Hat1[Δ57] mutants are viable and fertile, with a mild sublethal phenotype. We demonstrated that dHat1 has a H4K5 and H4K12 specific histone acetyltransferase activity. By immmuncytochemistry of Hat1[Δ57] and histone PTM mimic transgene His4r-K5R-K12R we showed, that this modification is not required for nuclear localization of H4 histones. As loss of Hat1 resulted in complete lack of H4K5 and H4K12 acetylation in embryos we analyzed gene expression in Hat1[Δ57] embryos by RNA-seq and found that it affected the transcript levels of ~2000 genes. As the role of B-type acetyltransferases in Huntington's disease was not tested before we investigated whether Hat1[Δ57] influences mutant Htt induced phenotypes and found that reduced Hat1 ameliorated mutant Htt induced neurodegeneration. [*manuscript prepared and ready for publication: Varga J, Korbai S, Neller A, Zsindely N, Bodai L. Hat1 acetyltransferase modulates transcription during embryogenesis in Drosophila.*]

**Investigations into the effects of green tea infusion on Htt pathology**

We also investigated whether green tea infusion, which is known to contain beneficial polyphenolic compounds impacts HD pathology. We found that green tea supplementation reduced mutant Htt induced neurodegeneration in *Drosophila* and positively impacted the longevity of mutant Htt expressing flies. However, green tea did not rescue reduced viability of *Drosophila* expressing mutant Htt. [*Varga J, Dér NP, Zsindely N, Bodai L. Green tea infusion alleviates neurodegeneration induced by mutant Huntingtin in Drosophila. Nutr Neurosci. 2018. Jul 4:1-7.*]

## Collaborative projects

We used the know-how we gained during the execution of this project to be involved in collaborative projects, some of which resulted in publications. We participated in the characterization of gene expression in different parts of the Drosophila testis [*Vedelek V, Bodai L, Grézal G, Kovács B, Boros IM, Laurinyecz B, Sinka R. Analysis of Drosophila melanogaster testis transcriptome. BMC Genomics. 2018. 19:697.*].

We also analyzed transcriptomic changes and transposon activation in *small ovary* mutants, and showed that sov is required for proper transposon silencing. [*Jankovics F, Bence M, Sinka R, Faragó A, Bodai L, Pettkó-Szandtner A, Ibrahim K, Takács Z, Szarka-Kovács AB, Erdélyi M. Drosophila small ovary gene is required for transposon silencing and heterochromatin organization, and ensures germline stem cell maintenance and differentiation. Development. 2018. 145. pii: dev170639.*]

## Scientific Training

In connection with the research project eight BSc theses, three MSc theses, and four Scientific Students' Associations papers were prepared.