enzymes in the context of ASD has not been explored yet. Future directions might involve the evaluation of a recently FDA-approved potent inhibitor of EZH2 (tazemetostat) to treat epithelioid sarcoma that should activate *EphA7* mRNA transcription similarly to UNC1999 (Figure 1). Although this avenue represents a potential therapeutic strategy in ASD, further studies are needed to determine their efficacy and to exclude possible side effects due to lack of specificity. Further, a window of opportunity for patient therapy might exist during synaptogenesis before brain wiring is completed.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Cheon, S., Culver, A.M., Bagnell, A.M., Ritchie, F.D., Vacharasin, J.M., McCord, M.M., Papendorp, C.M., Chukwurah, E., Smith, A.J., Cowen, M.H., et al. (2022). Counteracting epigenetic mechanisms regulate the structural development of neuronal circuitry in human neurons. Mol. Psychiatry. Published online February 24, 2022. https://doi.org/10.1038/s41380-022-01474-1.

Datson, N.A., van den Oever, J.M.E., Korobko, O.B., Magarinos, A.M., de Kloet, E.R., and McEwen, B.S. (2013). Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. Endocrinology *154*, 3261–3272.

Faust, T.E., Gunner, G., and Schafer, D.P. (2021). Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS. Nat. Rev. Neurosci. *22*, 657–673.

Gao, Y., Duque-Wilckens, N., Aljazi, M.B., Wu, Y., Moeser, A.J., Mias, G.I., Robison, A.J., and He, J. (2021). Loss of histone methyltransferase ASH1L in the developing mouse brain causes autistic-like behaviors. Commun Biol *4*, 756.

Herre, M., and Korb, E. (2019). The chromatin landscape of neuronal plasticity. Curr. Opin. Neurobiol. *59*, 79–86.

Lévy, J., Schell, B., Nasser, H., Rachid, M., Ruaud, L., Couque, N., Callier, P., Faivre, L., Marle, N., Engwerda, A., et al. (2021). EPHA7 haploinsufficiency is associated with a neurodevelopmental disorder. Clin. Genet. *100*, 396–404.

Qin, L., Williams, J.B., Tan, T., Liu, T., Cao, Q., Ma, K., and Yan, Z. (2021). Deficiency of autism risk factor ASH1L in prefrontal cortex induces epigenetic aberrations and seizures. Nat. Commun. *12*, 6589.

Satterstrom, F.K., Kosmicki, J.A., Wang, J., Breen, M.S., De Rubeis, S., An, J.-Y., Peng, M., Collins, R., Grove, J., Klei, L., et al.; Autism Sequencing Consortium; iPSYCH-Broad Consortium (2020). Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. Cell *180*, 568–584.e23.

Woldemichael, B.T., Bohacek, J., Gapp, K., and Mansuy, I.M. (2014). Epigenetics of memory and plasticity. Prog. Mol. Biol. Transl. Sci. *122*, 305–340.

Yan, Y., Tian, M., Li, M., Zhou, G., Chen, Q., Xu, M., Hu, Y., Luo, W., Guo, X., Zhang, C., et al. (2022). ASH1L haploinsufficiency results in autistic-like phenotypes in mice and links Eph receptor gene to autism spectrum disorder. Neuron *110*, 1156–1172.

Back-to-BACs in Huntington's disease modeling

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In this issue of *Neuron*, Gu et al. (2022) describe a new BAC mouse model that faithfully recapitulates various aspects of Huntington's disease pathobiology and reveals important insights into the relative toxicities of *mHTT*-derived products.

Despite the fact that the causative genetic mutation underlying Huntington's disease (HD) was identified as CAG trinucleotide repeat expansions in the huntingtin (*HTT*) gene in 1993, there is still no curative therapeutic for this fatal disease. *HTT* lowering as a therapeutic strategy has generated considerable recent interest, but it is now clear that successful and widespread application of *HTT* lowering strategies may be years away. Given this, there is still a need to understand basic mechanisms that underlie HD pathobiology and for better preclinical models for testing *HTT*-lowering therapeutics.

Perhaps more than for any other neurodegenerative disease, mouse models have yielded key insights into HD mechanisms and therapeutic opportunities. It was in the R6/2 model (Mangiarini et al., 1996) that intraneuronal nuclear inclusions (NIs) of mutant Huntingtin (mHTT) protein were first described (Davies et al., 1997), an important finding that then led to the identification of similar NIs in the human HD brain. Further, HD mouse models were used to show a gain-of-function toxicity for mHTT, as well as in pioneering proof-of-principle studies demonstrating the therapeutic effects of *HTT*-lowering strategies. Nevertheless, there exist limitations for all of the currently used HD mouse models. Widely used HD knockin models with human *mHTT* exon1 and high CAG repeat lengths (e.g., Q111, Q140, and derived lines [Menalled et al., 2003; Wheeler et al., 1999]) show striatum-selective mHTT aggregates and NIs,

Table 1. BAC-CAG phenotype overview (12 months of age)
Impaired performance on the accelerating rotarod, hypolocomotion, and reduced grip strength
Sleep disruption
Striatal synapse loss
Striatal and corpus callosum astrogliosis and striatal microgliosis
S830-antibody HTT diffuse nuclear staining and NIs in striatum (diffuse nuclear staining in cortex at 18 months)
Striatal-enhanced and age-dependent transcriptional dysregulation
Minimal weight gain versus controls
Somatic *mHTT* CAG repeat instability in striatum and liver
Aberrantly spliced *mHTT*-exon1 mRNA
mHTT/*Htt* mRNA (and Mbnl1+) foci in striatum and cortex
PolySerine RAN translation products in striatum and cortex (at 18 and 22 months of age)
Entire human *HTT* genomic locus
No overt brain atrophy or neuronal degeneration
No reported hindlimb clasping or cognitive phenotypes
No reported oxidative damage or mitochondrial dysfunction

CAG-length-dependent transcriptional dysregulation, and behavioral deficits. However, an important limitation of these models is that they lack human *HTT*-specific genomic regulatory elements as well as alternative splicing. For this reason, these models cannot be used to study therapeutics designed to induce *HTT*-lowering alternative splicing events or allele-selective lowering of *mHTT* (as they lack patient-associated SNPs), nor can they be used to study the impact of human *HTT* genomic elements outside of exon 1 that may regulate CAG somatic instability. Bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) models such as the BACHD (Gray et al., 2008) and YAC128 (Slow et al., 2003) models contain the entire human *HTT* genomic locus as transgenes, but because they were engineered to contain CAA-interrupted CAG repeats, they do not display CAG repeat instability in the germline or somatic tissues and are also limiting in studying the effects of *mHTT* RNA-associated toxicities. In addition, both the BACHD and YAC128 models display weight gain due to *HTT* overexpression, a feature that confounds certain types of behavioral testing, and these models importantly also show small amounts of NIs and transcriptional dysregulation. Finally, an important biological question that has been raised is whether it is the numerous species-specific sequence differences between the human *HTT* and murine *Htt* genes, or simply the fact that the transgenic BAC/YAC models contain CAA-interrupted CAG repeats, that accounts overall for the large phenotypic divergence of the knockin versus BAC/YAC HD mouse models. In this issue of *Neuron*, Gu et al. (2022) report a new BAC model of HD, the BAC-CAG model, that expresses *mHTT* with 120 uninterrupted CAG repeats (and about 131 total polyglutamine repeats), which combines important features of both the knockin and BAC/YAC models (Table 1), and that clearly shows that the presence of CAA-interrupted CAG repeats is what mostly accounts for the previously noted phenotypic divergence between the knockin and BAC/YAC models. With regard to therapeutics testing, the BAC-CAG model is a unique model that can be used to evaluate the therapeutic synergy of genetic or molecular therapies targeting human *HTT* as well as those targeting the CAG repeat instability.

Impaired motor coordination and locomotor activity are among the most commonly tested behaviors in HD model mice, as they reflect motor deficits in human HD patients. BAC-CAG mice show significant impairment on the accelerating rotarod test at 6 and 12 months of age, as well as grip strength at 12 months of age. In addition to motor impairments, BAC-CAG mice display reduced nighttime locomotor activities and more fragmented activity patterns, as well as reduced daytime sleep and sleep distribution, important features that model circadian and sleep disturbances in HD patients. An important feature of the new BAC-CAG model is that only male mice display a small amount of weight gain versus controls (~5%) at one age-point (12 months), likely due to the lower level of human *mHTT* overexpression that is present versus that present in other transgenic models.

To determine whether BAC-CAG mice also demonstrate HD-like neuropathology, the authors assessed brain atrophy, synapse loss, and gliosis. No overt brain atrophy was seen, unlike what is observed in the BACHD/YAC128 models, but loss of the synaptic marker *Actn2* and overt loss of dorsolateral striatal medium spiny neurons' spine density was noted at 12 months of age. Also at this age, there was evidence of astrogliosis in the striatum and corpus callosum and microgliosis in the striatum. Assessing for NIs in the BAC-CAG mice, the authors observed diffuse mHTT accumulation in a subset of striatal neurons at 12 months of age, which evolved into NIs in the majority of striatal neurons at 18 months of age. At 18 months of age, mHTT NIs and diffuse nuclear staining were also observed in a small number (5%–10%) of deep layer cortical pyramidal neurons. Further analyses indicated that aggregated HTT species increased in the striatum and cerebellum at 12 and 18 months of age, as well in the cortex at 18 months of age. Altogether these findings indicated that the BAC-CAG mice display earliest and highest mHTT aggregation in the striatum. And while these findings are consistent with the striatum-enhanced mHTT aggregation that is also seen in various *mHTT* knockin mice, they raise the interesting question of why no mouse model yet recapitulates the reported human HD brain neuropathology of NIs being most prevalent in the cortex (Gutekunst et al., 1999). However, it is important to note that these human studies were based on relatively few post mortem cases, and further human pathological studies may resolve this apparent discrepancy. When considering the cortical layer NI distribution, the deep layer distribution seen in the BAC-CAG model is more faithful to that seen in HD patients versus the distribution seen in the large CAG-length knockin model

mice (e.g., Q140/Q175), which have more upper cortical layer distribution.

Transcriptional dysregulation is often used as a molecular readout of HD model progression. In a series of similar mouse models differing mainly by CAG repeat number (the "allelic series" of *mHTT* knockin mice), transcriptional dysregulation is both age- and CAG-length dependent and is most pronounced in the striatum (Langfelder et al., 2016). Performing bulk RNA sequencing analysis of the striatum and cortex of BAC-CAG mice at 2, 6, and 12 months of age, Gu et al. observed an age-dependent and striatum-enhanced transcriptional dysregulation that included downregulation of striatal medium spiny neuron identity genes and genes reported to be downregulated in the remaining cells of human HD striatal samples. Further, the genes dysregulated at 12 months of age were very similar to those in a comparable-CAG-length knockin model (Q140 human *HTT* exon 1 knockin). Interestingly, however, this similarity was observed when comparing 12 month BAC-CAG mice to 6 month Q140 knockin mice, suggesting that some difference in models, perhaps in species difference in the HTT coding sequence and not only the pure CAG repeat number per se, can modulate when transcriptional dysregulation emerges.

Another important aspect of HD pathobiology is the somatic instability of the CAG repeat region in the *mHTT* locus, the slowing of which is currently being actively pursued as a therapeutic target. Somatic CAG instability was noted at 2 months of age in all tissues tested and was significantly increased at 12 months of age specifically in the striatum and liver in the BAC-CAG mice. Interestingly, the authors also found a significant negative correlation between *mHTT* CAG instability indices and locomotor activity (cortical instability) and nighttime sleep (striatal instability), showing clear links between *mHTT* somatic instability and behavior. Hopefully similar analyses will become standard in future studies that assess HD model phenotypes.

To address non-polyglutamine species that may lead to pathogenic effects in the BAC-CAG model, the authors also assessed *mHTT* transcripts and found evidence for the presence both of bidirectional (sense and antisense) and aberrantly spliced *mHTT*-exon1 mRNAs that have been reported in other HD mouse models and HD patient samples. They also identified *mHTT* and *Htt* RNA foci, as well as Mbnl1+ nuclear foci in the striatum and cortex of BAC-CAG mice at 12 months of age and found evidence for repeat-associated non-ATG (RAN; Bañez-Coronel et al., 2015) polySerine translation protein products in the striatum and cortex at 18 and 22 months of age.

By comparing the new BAC-CAG model with other models, the authors addressed the relative toxicities of the many abnormal features that may arise from the *mHTT* gene. Among all of the models studied, there was a strong positive correlation between the uninterrupted CAG repeat length (not just polyglutamine length) and concordance of striatal transcriptional dysregulation. Based on the spatiotemporal order of their emergence, as well as by comparison to other HD models' phenotypes, the authors hypothesize that *mHTT* CAG instability, *mHTT* exon1 transcripts and/or their encoded protein products, and Mbnl1+ foci accumulation may all contribute to the enhanced striatal vulnerability at the onset of disease phenotypes. Given their later-onset accumulation (18 and 22 months versus 12 months for most other findings), *mHTT* RAN translation-derived polySerine products were hypothesized to contribute to later stages of disease, although other RAN-derived products were not assessed in this study. Finally, the authors point to the fact that common cortico-striatal synaptic deficits can be found across pure- and interrupted-CAG HD models, indicating that this subset of the HD model phenotype can be mainly attributed to mHTT polyglutamine protein toxicity. While the study's findings do support a critical role of uninterrupted *mHTT* CAG repeat in eliciting striatum-selective HD-associated phenotypes, fully disentangling mHTT polyglutamine protein versus RNA-based toxicities will require future studies that focus on single-cell metrics, as undoubtedly per-cell differentially expanded polyglutamine protein species of potentially different toxicities will result from *mHTT* CAG somatic instability.

DECLARATION OF INTERESTS

Dr. Myriam Heiman is a member of the Hereditary Disease Foundation's Scientific Advisory Board.

REFERENCES

Bañez-Coronel, M., Ayhan, F., Tarabochia, A.D., Zu, T., Perez, B.A., Tusi, S.K., Pletnikova, O., Borchelt, D.R., Ross, C.A., Margolis, R.L., et al. (2015). RAN Translation in Huntington Disease. Neuron *88*, 667–677.

Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L., and Bates, G.P. (1997). Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell *90*, 537–548.

Gray, M., Shirasaki, D.I., Cepeda, C., André, V.M., Wilburn, B., Lu, X.-H., Tao, J., Yamazaki, I., Li, S.-H., Sun, Y.E., et al. (2008). Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J. Neurosci. *28*, 6182–6195.

Gu, X., Richman, J., Langfelder, P., Wang, N., Zhang, S., Bañez-Coronel, M., Wang, H.-B., Yang, L., Ramanathan, L., Deng, L., et al. (2022). Uninterrupted CAG repeat drives striatum-selective transcriptionopathy and nuclear pathogenesis in human Huntingtin BAC mice. Neuron *110*, 1173–1192.

Gutekunst, C.A., Li, S.H., Yi, H., Mulroy, J.S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R.J., Hersch, S.M., and Li, X.J. (1999). Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J. Neurosci. *19*, 2522–2534.

Langfelder, P., Cantle, J.P., Chatzopoulou, D., Wang, N., Gao, F., Al-Ramahi, I., Lu, X.-H., Ramos, E.M., El-Zein, K., Zhao, Y., et al. (2016). Integrated genomics and proteomics define huntingtin CAG length-dependent networks in mice. Nat. Neurosci. *19*, 623–633.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W., and Bates, G.P. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell *87*, 493–506.

Menalled, L.B., Sison, J.D., Dragatsis, I., Zeitlin, S., and Chesselet, M.-F. (2003). Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. J. Comp. Neurol. *465*, 11–26.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., et al. (2003). Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum. Mol. Genet. *12*, 1555–1567.

Wheeler, V.C., Auerbach, W., White, J.K., Srinidhi, J., Auerbach, A., Ryan, A., Duyao, M.P., Vrbanac, V., Weaver, M., Gusella, J.F., et al. (1999). Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Hum. Mol. Genet. *8*, 115–122.