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# Huntingtin-Lowering Therapies for Huntington Disease
## A Review of the Evidence of Potential Benefits and Risks

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Huntington disease (HD) is caused by a cytosine-adenine-guanine trinucleotide repeat expansion in the huntingtin gene, *HTT*, that results in expression of variant (mutant) huntingtin protein (HTT). Therapeutic strategies that reduce HTT levels are currently being pursued to slow or stop disease progression in people with HD. These approaches are supported by robust preclinical data indicating that reducing variant huntingtin protein is associated with decreased HD pathology. However, the risk-benefit profile of reducing either variant HTT or both variant and wild-type HTT is currently an open question that is being addressed in ongoing clinical trials. This review aims to examine the current data available regarding altered *HTT* in humans, normal animals, and animal models of HD. Studies indexed in PubMed were searched using the MeSH term *Huntington disease* or the text words *huntington* or *huntingtin* from August 31, 1999, to August 31, 2019, with no language restrictions. Additional studies were included from the reference lists of relevant studies and the authors' personal files. Articles describing at least 1 aspect of HTT reduction were included, prioritizing those published within the last 10 years. In vivo studies were also prioritized, with a focus on studies that examined the consequences of wild-type HTT reduction in adults. In a recently completed phase 1/2a study of RG6042 in 46 adults with early manifest HD, antisense oligonucleotide-mediated partial reduction of HTT was reported to be generally safe and well tolerated over the course of 4-monthly RG6042 doses. In case studies of people with rare genetic variations in huntingtin alleles, the loss of 1 wild-type allele was not associated with HD. People with homozygous cytosine-adenine-guanine expansions developed normally until the onset of HD, although they may have experienced a more aggressive disease course. In mouse models of HD, partial reduction of HTT was beneficial, with improvements in motor, cognitive, and behavioral phenotypes. The partial reduction of wild-type HTT in normal adult rodents and nonhuman primates was generally safe and well tolerated. The body of evidence reviewed in this article indicates a positive risk-benefit profile for the partial reduction of either variant HTT alone or both variant and wild-type HTT. These strategies target the underlying cause of HD and are currently being tested in several investigational clinical trials.

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Huntington disease (HD; OMIM 613004) is a rare genetic neurodegenerative disease characterized by a triad of cognitive, behavioral, and motor symptoms.[1-3] Disease onset typically occurs in the prime of life, between ages 30 and 50 years, and is associated with increasing disability, worsening of function, and loss of independence, leading to death within approximately 15 years, on average, after the onset of motor signs and symptoms.[2,4] A cytosine-adenine-guanine (CAG) repeat expansion variant (mutation) in only 1 of the 2 alleles of the huntingtin gene, *HTT* (OMIM 613004), is sufficient to be associated with the onset of HD with an autosomal-dominant pattern of inheritance.[5] The expansion variation on the affected allele is encoded for an abnormally long polyglutamine tract within the huntingtin protein (HTT), resulting in the formation of variant HTT.[1,6,7] The expression of variant HTT throughout the brain is associated with progressive age-dependent neurodegeneration, primarily owing to toxic gain-of-function mechanisms.[1,8,9] Strategies to decrease or suppress the production of variant HTT are in clinical development, with the ultimate goal of stopping or slowing clinical progression of the affected cognitive, behavioral, and motor domains.[1,7] These strategies include antisense oligonucleotide (ASO)–mediated HTT-lowering approaches, which target the RNA product of either the variant *HTT* allele or both *HTT* alleles (the variant *HTT* and the unaffected and unexpanded or wild-type *HTT* allele) as well as adeno-associated viral (AAV) vector–delivered short interfering RNA (siRNA) or microRNA (miRNA) HTT-lowering approaches, which target the products of both *HTT* alleles.

This review examines all available evidence from the relevant published human and animal literature to assess the potential benefits and risks of HTT-lowering therapeutic strategies in clinical development for the treatment of HD (**Figure**).[2,10-28] The huntingtin protein is large and ubiquitously expressed (3144 amino acids), with



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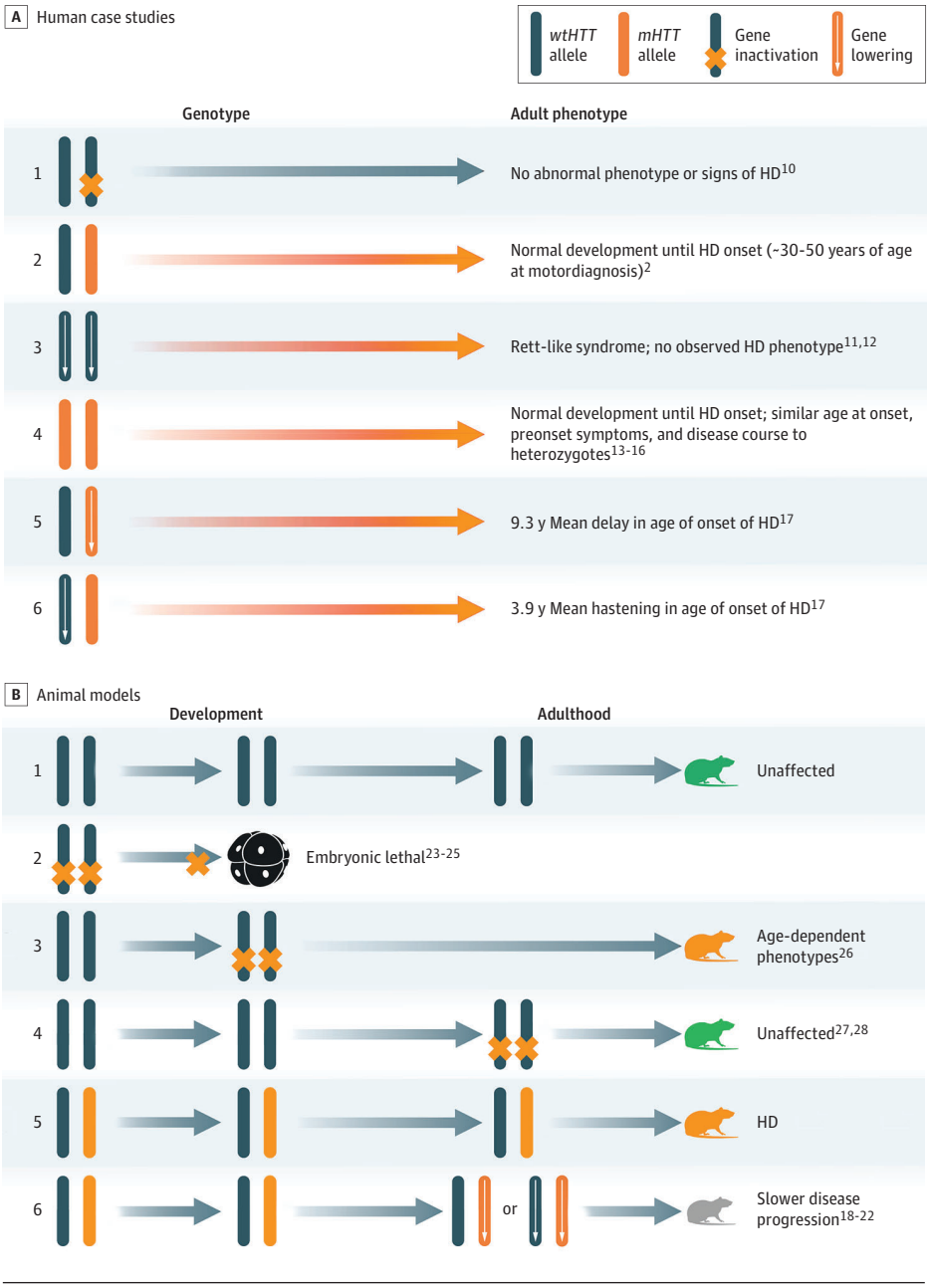
Figure. Consequences of Reducing HTT




A, Row 1 represents normal development. Row 2 represents genetic ablation of wild-type HTT from conception, which is lethal in the embryonic stage because wild-type HTT is required for central nervous system development. Row 3 represents rare compound heterozygous variations in both *HTT* alleles, which are likely to have less than 50% of normal HTT function. Row 4 represents wild-type HTT inactivation during adulthood, which does not appear to be associated with HD symptoms, with a phenotype similar to those with 2 wild-type *HTT* gene copies. Row 5 represents a single copy of the variant *HTT* gene, which is associated with the development of HD. Row 6 represents partial reduction of either variant HTT alone or combined wild-type and variant HTT, which, in adulthood, can slow disease progression and delay the onset of HD symptoms. B, Row 1 represents normal development. Row 2 represents genetic ablation of wild-type HTT from conception, which is lethal in the embryonic stage because wild-type HTT is required for central nervous system development. Row 3 represents wild-type HTT inactivation during late stages of development, which does not produce the HD-like phenotype but is associated with other age-dependent central nervous system phenotypes. Row 4 represents wild-type HTT inactivation during adulthood, which does not appear to be associated with HD symptoms, with a phenotype similar to those with 2 wild-type *HTT* gene copies. Row 5 represents a single copy of the variant *HTT* gene, which is associated with the development of HD. Row 6 represents partial reduction of either variant HTT alone or combined wild-type and variant HTT, which, in adulthood, can slow disease progression and delay the onset of HD symptoms.

multiple pleiotropic functions, including important roles in synaptic function, transcriptional regulation, and vesicular transport.[9] The huntingtin protein is also important in neurodevelopment (ie, the absence of HTT is lethal in the embryonic stage).[8,9,29] In accordance, the age at and degree to which HTT-lowering strategies will be beneficial and tolerated in the clinic, and when or where such reductions will be beneficial and tolerated during the course of HD, are currently unknown. Drug-related differences in the approaches being pursued further complicate the issue. Whereas ASO-mediated HTT-lowering approaches are partial and reversible manipulations of HTT that primarily target cortical rather than subcortical structures, AAV-delivered siRNA or miRNA approaches are permanent and irreversible and primarily target subcortical structures.

The available data suggest that targeting variant HTT is likely to be essential for any treatment of the underlying mechanisms associated with HD, that at least 50% of normal HTT function is sufficient for normal development, and that a greater than 50% reduction in HTT during early development may be detrimental.[30] However, the risk-benefit ratio of decreasing HTT in adults with HD as a function of the therapeutic strategy chosen is unclear, and this risk-benefit ratio is the primary focus of this review, as adults constitute the target population for current clinical trials focusing on HTT reduction.



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Table 1. Studies of Altered Huntingtin Gene Levels in Humans

| Source | Genotype | Notes |
|---|---|---|
| Phase 1/2a clinical trial of RG6042[31] | Wild-type HTT and variant HTT reduction with 4 monthly doses of RG6042 | Generally safe and well tolerated |
| Case study[10] | Woman with 1 normal and 1 disrupted *HTT* allele | No detectable abnormal phenotype at age 46 y |
| Case series[11,12] | Children with compound heterozygous HTT variations and likely decreased HTT function | Early neurodevelopmental disorder with features of Rett-like syndrome but no HD phenotype |
| Case series[13-16] | Homozygous CAG-expansion variations | Normal development until onset of HD |
| | | No association with age at onset and preonset |
| Epidemiology study[17] | Transcription-lowering variants of either variant *HTT* or wild-type *HTT* alleles | Reduction of variant HTT associated with delayed HD onset by a mean of 9.3 y |
| | | Reduction of wild-type HTT associated with hastened HD onset by a mean of 3.9 y |

Abbreviations:
CAG, cytosine-adenine-guanine;
HD, Huntington disease;
*HTT*, huntingtin gene;
HTT, huntingtin protein.

## Approaches and Observations

We searched studies indexed in PubMed using the MeSH term *Huntington disease* or the text words *huntington* or *huntingtin* from August 31, 1999, to August 31, 2019, with no language restrictions. We identified additional studies by reviewing the reference lists of relevant studies and searching our personal files. In our initial analysis, we included all articles that described at least 1 aspect of HTT reduction and prioritized those published within the last 10 years. The evidence reviewed included published primary reports of studies that were relevant to altered wild-type HTT levels in humans, partial wild-type HTT-lowering therapies in normal rodents, nonhuman primate and transgenic rodent models of HD, genetic inactivation of wild-type HTT in otherwise normal rodents, genetic manipulation of wild-type HTT in transgenic rodent models of HD, and available data from HTT-lowering clinical trials in humans.

Because the HTT-lowering therapies currently in development are designed for the treatment of adults with HD, in vivo studies were prioritized for review, with a focus on the consequences of wild-type HTT reduction in adult animals and humans. Studies of the consequences of the genetic inactivation of wild-type HTT during embryogenesis were not directly relevant to this therapeutic strategy but have been included for completeness. Primary literature on in vitro studies was not included because these studies had less potential relevance to the treatment of HD, although relevant reviews within the last 10 years were cited, and the relevance of key in vitro findings to the presented data are discussed in this review.

### HTT Levels in Humans

**Table 1** summarizes the findings of studies that have examined the consequences of HTT reduction in adult humans. The recently completed phase 1/2a clinical trial of RG6042 in 46 adult patients with HD provides the most direct evidence on the safety of ASO-mediated HTT reduction.[31] Treatment with RG6042 was generally safe and well tolerated over the course of 4-monthly doses up to the highest dose tested, and no patients discontinued treatment in the study. These results indicate that ASO-mediated partial HTT reduction is a viable clinical strategy in patients with HD, and further assessment is ongoing in an open-label extension of the completed phase 1/2a clinical trial and in the ongoing 25-month GENERATION HD1 study of patients with manifest HD.[32,33]

Studies of the potential consequences of reduced HTT levels in humans have historically relied on identifying rare cases of people with naturally occurring *HTT* variations. In contrast with ASO-based HTT-lowering therapies for adults with HD, genetic differences in *HTT* expression are associated with altered HTT levels at conception and throughout life. Ambrose et al[10] described a woman with 1 intact and 1 disrupted *HTT* allele that could, at best, express a truncated protein product. This heterozygous inactivation of *HTT* was not associated with any detectable abnormal phenotype at age 46 years. Both the woman and 1 of her children, who also carried the disrupted *HTT* allele, were phenotypically normal and did not exhibit any signs of HD or aberrant development.[10] These findings indicate that a heterozygous reduction in HTT levels is not associated with the onset of HD and that 50% of the normal levels of HTT are compatible with normal development and brain function. A potential caveat to our interpretation of this study is that the absolute levels of HTT protein in this family were not reported, and our interpretation is based on the presence of a hemizygous null deletion of the *HTT* gene.

In other individuals with rare compound heterozygous variations (putative hypomorphic or loss-of-function variants) in both *HTT* alleles, which are estimated to have less than 50% of normal HTT function throughout embryonic development, neurodevelopmental delay and an associated Rett-like clinical syndrome were observed, but no evidence of an HD phenotype was found.[11,12] These clinical findings were mirrored in hypomorphic mice expressing decreased HTT levels, in which severe HTT loss (less than 5% of wild-type HTT levels) was associated with lethality in the early embryonic stage, while less severely reduced HTT (less than 20% of wild-type HTT levels) was associated with various neurodevelopmental abnormalities.[34]

In multiple case series of people with HD who have rare homozygous CAG-expansion variations in *HTT* (2 variant *HTT* alleles and no wild-type *HTT* alleles), development was normal until the onset of HD, with age at onset and preonset symptoms similar to those of people with heterozygous HD (1 wild-type *HTT* allele and 1 variant *HTT* allele).[13-16] Furthermore, in most cases, the clinical disease course and progression were not different than those of individuals with heterozygous HD.[35] These findings indicate that variant HTT functions normally during development and that the polyglutamine expansion is not primarily a loss-of-function variation.

A clinical and genetic HD epidemiological study by Becanovic et al[17] investigated the clinical associations between the presence



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Table 2. Partial Reduction of Huntingtin Gene in Animal Models of Huntington Disease

| Treatment | HTT reduction[a] | Model | Benefits | Notes |
|---|---|---|---|---|
| **Reversible reduction** | | | | |
| MoHuASO[18] | Variant HTT and wild-type HTT (approximately 75% maximum; normalization after 4 mo; mRNA) | BACHD mouse ($Hdh^{+/+}$) | Improved motor coordination | No attenuation of beneficial functional associations vs variant HTT-specific ASO |
| | | | Normalized hypoactivity | |
| Allele-specific (variant HTT1 or variant HTT3) or HTT-lowering (hHTT)[19] | Variant HTT maximum protein reduction: 89% variant HTT1; 71% variant HTT3; 78% hHTT | Hu97/18 | Improved cognition and behavioral phenotype across all studied ASOs | Potency of ASO on variant HTT reduction appears to be associated with more improvement across experiments between the 3 studied ASOs |
| **Nonreversible reduction** | | | | |
| AAV-mi2.4[20] | Variant HTT and wild-type HTT (60% at 4 wk, 75% at 4 mo; mRNA) | HD-N171-82Q mouse ($Hdh^{+/+}$) | Improved motor function | None |
| | | | Likelihood of longer life span | |
| LV-sihtt6[21] | Variant HTT and wild-type HTT (65%-75% at 13 wk persisting to 9 mo; mRNA) | Wistar rat ($Htt^{+/+}$)[b] | Increased GABAergic neuronal survival and reduced inclusion load | No difference in GABAergic neuronal survival vs variant HTT reduction alone |
| | | | | No signs of toxic effects |
| LV-sihtt13[21] | Wild-type HTT only (65%-75%; mRNA) | Wistar rat ($Htt^{+/+}$)[b] | None (no variant HTT reduction) | No difference in GABAergic neuronal survival or inclusion load vs untreated |
| | | | | No signs of toxic effects |
| AAV2/1-miRNA-Htt[22] | Variant HTT and wild-type HTT (55% at 12 wk; mRNA and protein) | YAC128 mouse ($Hdh^{+/+}$) | Improved motor function | No overt striatal toxic effects or neuroinflammation in histopathologic specimens |
| | | | Improved depressive-like behavior | |
| | | | Partial correction of aberrant striatal transcriptional profile | |
| | | | Reduced HTT aggregates | |

Abbreviations: AAV, adeno-associated virus; ASO, antisense oligonucleotide; GABAergic, γ-aminobutyric acid–mediated; HD, Huntington disease; HTT, huntingtin protein; LV, lentivirus; miRNA, microRNA; MoHuASO, mouse-human ASO.

[a] Quoted times are postadministration.

[b] Rats received intracortical injections of lentivirus-expressing variant HTT N171-82Q.

of a transcription-lowering variant located on either the variant *HTT* allele or the wild-type *HTT* allele and the age at HD onset. The presence of this transcription-lowering variant on the variant *HTT* allele was associated with a delay in the age at onset. Its presence on the wild-type *HTT* allele in a different cohort was associated with a younger age at onset, although this significant finding was not replicated in a second and less well-characterized independent cohort. The *HTT* transcription-lowering variant was identified as a noncoding single nucleotide substitution in a transcription factor–binding site in the *HTT* promoter that was associated with reducing transcriptional activity by approximately 50% in vitro.[17] These results are the first definitive human data to suggest that decreased levels of variant HTT are associated with less severe disease and that HTT reduction should have net benefits in people with HD. These data also raise some concerns about decreasing the levels of wild-type HTT when the levels of variant HTT are not reduced. It is important to recognize that these genetic changes in HTT expression are present throughout life, including during important neurodevelopmental periods.

## HTT Levels in Animals

**Table 2** summarizes the studies of HTT reduction in animal models of HD, including reversible ASO-based or irreversible long-term AAV vector–based approaches. In all studies, HTT reduction was associated with beneficial consequences in animals with HD-like disease, with no exacerbation or worsening of disease progression. Moreover, in direct comparisons, the reduction of both wild-type HTT and variant HTT was associated with benefits similar to variant HTT reduction alone. With the exception of the fully humanized mouse model, Hu97/18, the HD models used in these studies involved the expression of a single variant *HTT* transgene in rodents carrying 2 wild-type copies of the endogenous *HTT* ortholog (designated $Hdh^{+/+}$ in mice or $Htt^{+/+}$ in rats).[36]

The administration of a mouse-human ASO targeting both variant HTT in humans and wild-type HTT in mice was associated with a transient decrease of approximately 75% in the whole-brain levels of both proteins in BACHD mice.[18] The motor coordination of the mice improved on rotarod tests for 3 to 8 months after ASO infusion into the cerebrospinal fluid (CSF) compared with mice who did not receive ASO infusion. In open-field tests, hypoactivity normalized by 5 months after treatment. The extent of these improvements was similar to that observed with the reduction of human variant HTT alone, indicating that wild-type HTT reduction did not attenuate the benefits of variant HTT reduction in the BACHD mouse model.[18] The same study also reported delayed disease progression, sustained phenotypic reversal, and increased life span in R6/2 and YAC128 mice that received variant HTT-specific ASO treatment.[18]

Treatment with an ASO targeting both wild-type *HTT* and variant *HTT* in mice has been tested in the fully humanized Hu97/18 transgenic mouse model of HD alongside treatment with variant *HTT*-specific ASOs (variant HTT1 and variant HTT3).[19] The administration of the human HTT ASO was associated with a 78% maximum reduction in variant HTT levels, decreasing to approximately 40% at 6 months after administration. The variant HTT1 ASO was slightly more potent, with an 89% maximum reduction in variant HTT levels and an approximate 60% reduction at 6 months after administration. The treatment of Hu97/18 mice with any ASO at age 6 months mitigated cognitive deficits and normalized anxiety-like and depres-



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Table 3. Partial Reduction of Wild-Type HTT in Normal Adult Nonhuman Primates

| Treatment | Magnitude of wild-type HTT reduction, % | Follow-up duration | Notes |
|---|---|---|---|
| **Reversible reduction** | | | |
| MkHuASO[18] | 32-54 (mRNA) | 8 wk | Sustained wild-type HTT reduction in most brain and spinal cord regions |
| siHtt[38] | 44 (mRNA) | 28 d | No behavioral changes noted |
| | 32 (protein) | | No abnormal histopathologic findings |
| **Nonreversible reduction** | | | |
| AAV2-HD5 shRNA[39] | 28-29 (mRNA) | 6 mo | No alterations in motor function |
| | 45 (protein) | | No abnormal histopathologic findings |
| AAV1-miHDS1[40] | 45 (mRNA) | 6 wk | No alterations in motor function |
| | | | No abnormal histopathologic findings |

Abbreviations: AAV, adeno-associated virus; ASO, antisense oligonucleotide; mRNA, messenger RNA; MkHuASO, monkey-human ASO; shRNA, short hairpin RNA; HTT, huntingtin protein.

sive behaviors at 3 months and 6 months after administration. The forebrain weight, cortical volume, striatal volume, and density of immunohistochemical staining for dopaminergic striatal neurons were substantially protected by the variant HTT-specific ASO and partially protected with human HTT at 6 months after administration. Although functional improvements were similar with HTT reduction compared with selective variant HTT reduction, subtle differences in the extent of benefits were observed in biochemical and neuroanatomical parameters. These differences may be associated with either the differences in potency and duration of action of the ASOs tested or the reduction of both wild-type HTT and variant HTT. Further experiments with matched levels of variant HTT suppression are needed to confirm these findings.

The administration of an AAV miRNA vector (AAV1-mi2.4) was associated with a reduction in both variant *HTT* and wild-type *Hdh* striatal messenger RNA (mRNA) levels of approximately 60% at 4 weeks and approximately 75% at 20 weeks after intrastriatal injection in HD-N171-82Q mice compared with injected control animals.[20] After treatment at age 7 weeks, statistically significant improvements in rotarod performance were observed at age 14 and 18 weeks compared with control animals. No safety findings of concern were noted, although rotarod performance, body weight, and life span were the only parameters reported.[20]

Lentivirus short hairpin RNA (shRNA) vectors have been used to investigate the consequences of wild-type HTT and variant HTT reduction in a rat model of HD.[21] Rats receiving intrastriatal injections of lentivirus-expressing human variant HTT (httN171-82Q) developed intraneuronal inclusions and had fewer striatal neurons after 2 months compared with rats that did not receive injections; motor function was not assessed. This pathology was decreased by variant HTT reduction via the coinjection of lentivirus shRNA vectors that specifically targeted human variant HTT or both human variant HTT and endogenous rat wild-type HTT, with no differences in striatal neuronal survival between the 2 approaches at 9 months after lentivirus infection. The coinjection of a lentivirus shRNA vector that targeted only wild-type HTT (and not variant HTT) in rats neither prevented nor exacerbated striatal neuropathology at 2 months after lentivirus infection. These findings led the authors to report that a 65% to 75% reduction of wild-type HTT expression in adult striatal neurons was well tolerated for up to 9 months in rodents.[21]

Treatment with an AAV miRNA vector was associated with a 55% decrease in both variant *HTT* and endogenous mouse wild-type *HTT* mRNA levels in the striatum of YAC128 mice when measured 1 month and 5 months after intrastriatal injection.[22] No remarkable histopathologic changes or signs of neuroinflammation were detected in the injected brain region at 5 months after lentivirus infection. Compared with untreated mice, treated mice exhibited improved motor function on rotarod tests and improved depressive-like behavior in forced swim tests, with performance indistinguishable from that of littermates with wild-type *HTT* at 2 to 3 months after lentivirus infection. Behavioral tests found no obvious adverse issues. Intraneuronal HTT aggregates were reduced, and a partial correction of the aberrant striatal transcriptional profile of YAC128 mice was also observed. The authors reported that partial reduction of endogenous mouse wild-type HTT in the central nervous system of YAC128 mice was well tolerated for up to 5 months.[22]

## HTT in Normal Animals

### Primates

To study the role of partial wild-type HTT-lowering interventions in adult animals, the administration of ASOs or siRNA via CSF infusion for short-term reductions and the delivery of AAV vectors of miRNA or shRNA via intracerebral injection for long-term reductions have been pursued in nonhuman primates and rodents.[36,37] **Table 3** summarizes studies of partial endogenous wild-type HTT reduction in nonhuman adult primates. Two studies evaluated transient wild-type HTT reduction using either ASO infusion into the CSF or siRNA infusion into the putamen. Three studies evaluated the injection of viral vectors expressing shRNA or miRNA into the caudate or putamen for long-term reduction. All interventions were associated with decreases in monkey wild-type HTT through the targeting of endogenous *HTT* mRNA in wild-type adult rhesus macaques.

Treatment with a monkey-human ASO was associated with decreases in endogenous wild-type *HTT* mRNA levels of 32% to 47% in the cortex and 54% in the spinal cord at 4 weeks after a 21-day continuous infusion into the CSF, with reductions still evident at 8 weeks.[18] Motor function and histopathology were not assessed. In unpublished studies of RG6042 conducted in nonhuman primates, including a chronic 9-month 10-dose study and a study of an ASO that targeted monkey *HTT* (ISIS 444652), no safety findings of concern associated with HTT reduction were identified (T. Zanardi, PhD, Director of Toxicology, Ionis Pharmaceuticals, written communication, February 25, 2020) (eTable 1 in the Supplement).

The administration of siRNA *Htt* was associated with a 44% decrease in endogenous wild-type *HTT* mRNA levels and a 32% decrease in wild-type HTT levels in the putamen of rhesus monkeys



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Table 4. Partial Reduction of Wild-Type HTT in Normal Adult Rodents

| Treatment | Magnitude of wild-type HTT reduction, % | Follow-up duration, mo | Mouse strain | Notes |
|---|---|---|---|---|
| **Reversible reduction** | | | | |
| MoHuASO[18] | 75 (mRNA) | 3-4 | FVB/N | No alterations in motor coordination or activity |
| **Nonreversible reduction** | | | | |
| LV-sihtt6[21] | 55 (mRNA) | 9 | C57/BL6 | Altered striatal gene expression |
| LV-sihtt13[21] | | | | |
| AAV2/1-miRNA-Htt[22] | 45 (mRNA) | 2-5 | FVB/NJ | No alterations in motor performance or activity |
| | 55 (protein) | | | No neuronal loss or neurotoxic effects in histologic analyses |
| AAV-mi2.4.GFP[41] | 70 (mRNA) | 4 | NR | Minimal striatal toxic effects in histologic analysis |
| AAV-mi2.4[20] | 65 (mRNA) | 4 | C57/BL6 | Altered striatal gene expression |

Abbreviations: AAV, adeno-associated virus; ASO, antisense oligonucleotide; GFP, green fluorescent protein; LV, lentivirus; mRNA, messenger RNA; miRNA, microRNA; MoHuASO, mouse-human ASO; NR, not reported; HTT, huntingtin protein.

after a 7-day continuous infusion into the striatum.[38] Histopathologic examinations after a 28-day continuous infusion revealed that tissue responses were confined to the catheter track and adjacent tissue, with no indication of neuronal loss elsewhere. The infusions were described as well tolerated, and no behavioral changes were observed (although no specific testing was described).

The administration of an AAV shRNA vector (AAV2-HD5) was associated with a 28% to 29% decrease in endogenous wild-type *HTT* mRNA levels and a 45% decrease in wild-type HTT levels in the striatum of rhesus monkeys at 6 months after injection into the caudate nucleus.[39] Compared with controls, no differences were noted in body weight, food consumption, or motor function (including forelimb performance, motor memory preservation, and daytime and nighttime home-cage activity levels), and histopathologic examinations revealed no discernible neuronal loss or abnormal astrocytosis.[39]

The injection of an AAV miRNA vector (AAV1-miHDS1) was associated with a 45% decrease in endogenous wild-type *HTT* mRNA levels in the putamen of rhesus monkeys at 6 weeks after injection into the putamen.[40] Compared with controls, no differences were noted in body weight, motor function (including forelimb fine motor skills and daytime and nighttime home-cage activity levels), and histopathologic examinations revealed no discernible neuronal loss, gliosis, or inflammation.[40] The performance of additional nonhuman primate studies with longer duration and greater HTT reduction will provide additional data regarding the long-term tolerability of HTT reduction in the adult brain.

### Rodents

**Table 4** summarizes studies of partial endogenous wild-type HTT reduction in normal adult rodents, with follow-up for 2 to 5 months. Four studies evaluated the intrastriatal injection of viral vectors expressing shRNA or miRNA, and 1 study evaluated the infusion of an ASO into the CSF. All interventions aimed to decrease mouse wild-type HTT levels by targeting endogenous *Hdh* mRNA in normal adult mice.

Treatment with a mouse-human ASO was associated with transient and reversible decreases in endogenous wild-type *HTT* mRNA levels in normal adult FVB/N mice, with a 75% reduction at 4 months and recovery of normal levels by 11 months after infusion into the CSF. Motor coordination and motor activity, which were measured by rotarod and open-field tests, were unimpaired at 3 months compared with untreated mice.[18]

Endogenous striatal wild-type *HTT* mRNA levels were reduced by approximately 55% in adult C57/BL6 mice after intrastriatal injection of lentivirus shRNA vectors (LV-sihtt6 and LV-sihtt13).[21] Striatal wild-type HTT reduction was associated with substantial changes in the expression of specific genes in the striatum according to transcriptomic analyses. The functions of the differentially expressed genes indicated roles in synaptic function, axonal guidance, and intracellular signaling pathways. The authors considered these roles to be associated with the known functions of HTT but indicated that further studies were needed to establish the potential consequences of the lentivirus shRNA-induced gene expression changes; phenotypic changes were not assessed.[21]

The administration of an AAV miRNA vector (AAV2/1-miRNA-Htt) was associated with a 45% to 55% decrease in endogenous striatal wild-type HTT mRNA and protein levels in adult FVB/NJ mice after intrastriatal injection.[22] No differences in motor performance in rotarod tests or motor activity in swim tests were detected at 2 to 3 months after injection, and histologic analyses revealed no signs of neuronal loss or neurotoxic effects at 5 months after injection.[22] The injection of similar AAV miRNA and shRNA vectors (AAV-mi2.4 green fluorescent protein [GFP] and AAV-sh2.4 GFP) was associated with a reduction in striatal wild-type HTT levels of approximately 70% at 4 months after injection in adult mice, and AAV-mi2.4 GFP was not associated with increased striatal neurotoxic effects compared with a mismatched control vector that did not alter wild-type HTT levels.[41] In a separate study, transcriptomic analyses indicated alteration of striatal gene expression after intrastriatal injection of AAV-mi2.4, with upregulation and downregulation of genes involved in multiple cellular processes, in which HTT also has associated roles (according to functional gene annotation clustering analyses).[20] The authors suggested that further investigation was necessary to determine whether these alterations were associated with changes in neuronal function.[20] Genetic ablation studies examining the inactivation of *Hdh* in different tissues or at different points in the life span of otherwise normal mice are summarized in the eAppendix, eTable 2, eTable 3, and eTable 4 in the Supplement.

Taken together, these studies of nonhuman primates and rodents suggest that partial reduction of wild-type HTT in adult



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animals is not detrimental; therefore, partial reduction of HTT levels may be a safe therapeutic strategy in human adults with HD.

## Discussion and Conclusions

Although HTT is an essential protein for embryogenesis and early brain development,[23-26,42-44] both wild-type HTT and variant HTT can support apparently normal development into adulthood in mice and humans, even when expressed from a single functional allele.[10,13-16,24] The HD or HD-like phenotype only develops across species when 1 or more CAG-expanded variant allele is present and expressed. These observations support the hypothesis that HD is not associated with a lack of or reduction in normal HTT function but rather with the neurotoxic gain-of-function effects of variant HTT. The similar therapeutic benefits of both variant HTT-specific and HTT-lowering interventions in the BACHD mouse and the humanized Hu97/18 mouse, which expressed only variant and normal transgenic human HTT, support this interpretation.[18,19,45]

Genetic ablation studies conducted during development, in which the *HTT* gene is 100% silenced, differ fundamentally from the clinical situation in adults with HD who receive partial HTT-lowering therapies, in which the HD phenotype is present and reductions in HTT levels are partial, reversible, titratable, and occur in the adult context. When genetic ablation of *Hdh* has been performed in rodents during early adulthood, this experimental condition has resulted in reported associations that are inconsistent and do not recapitulate the motor abnormalities or neuropathologic features that characterize HD in humans and animal models.[27,28] These experimental settings may nonetheless be relevant to studies that examine the complete nonreversible silencing of HTT expression.

Some murine studies have suggested that the presence of variant HTT may induce neurotoxic effects secondary to decreased HTT function during neurodevelopment,[46] which implies that HTT reduction may be well tolerated in the adult brain but may not be safe in the developing brain. In all clinical trials of HTT-lowering therapies, the timing of treatment is an important issue to consider. We recommend that these approaches not be pursued in children and adolescents without considering further information about the potential risks in the adult context to inform such efforts.

The potential consequences of large and persistent reductions in wild-type HTT levels are important theoretical risks for all potential HTT-lowering approaches to the treatment of HD that cross the 50% reduction threshold. The most compelling evidence to support this concern is the worsening of HD-like disease when wild-type *Hdh* is completely genetically inactivated from conception in a key transgenic mouse model of HD (YAC128), leaving variant transgenic *HTT* as the sole functional allele throughout the entire life span.[47] However, this experimental setting again fundamentally differs from the clinical situation in humans with HD, in which 2 functional *HTT* alleles are present from conception (1 variant and 1 normal), and HTT-lowering treatment is initiated in adulthood. Initial results from studies of RG6042, an ASO-mediated approach for the partial reduction of both wild-type and variant HTT, support this interpretation. Treatment with RG6042 was reported to be safe and well tolerated in all animal experiments to date and in humans with HD in the recent phase 1/2a clinical study.[18,19,31]

Although the intrathecal administration of RG6042 in patients with early HD was not accompanied by serious adverse events in the first-in-human study, an increase in CSF neurofilament light (NFL) chain protein and in ventricular volume without corresponding changes in whole-brain volume were observed. For those patients in whom NFL elevations were observed, no association with ventricular volume changes was identified, and values had returned to prestudy baseline levels at the beginning of the ongoing open-label extension of the phase 1/2a study.[31] In the ongoing open-label extension study, NFL levels increased again, then decreased almost to baseline by month 9 in the context of ongoing HTT suppression, without association with adverse or serious adverse events.[48]

The putative underlying mechanisms and ultimate clinical associations of these changes are presently unknown. The biphasic curve of the NFL changes observed (ie, a delayed increase of NFL in the CSF followed by a decrease of levels with continued treatment) suggests a response associated with distinct underlying component mechanisms. The putative underlying mechanisms include cellular responses through transcriptional changes, cellular structural reorganization, and/or transient cellular damage in vulnerable neuronal cell populations. These mechanisms may be associated with on-target wild-type HTT and/or variant HTT reduction, off-target response to ASO exposure independent of on-target wild-type HTT and/or variant HTT reduction, or a combination of both on-target and off-target changes. These diverse candidate mechanisms are being examined in preclinical and clinical investigations, including in the ongoing phase 3 randomized placebo-controlled GENERATION HD1 clinical trial, and these further studies will together identify the ultimate clinical associations and underlying mechanisms of these observed biomarker findings.

Concerns about the potential safety of HTT reduction are mitigated by the partial and reversible nature of this intervention. Antisense oligonucleotide–mediated HTT-lowering approaches are partial and reversible manipulations of HTT that primarily target cortical rather than subcortical structures, whereas AAV-delivered siRNA or miRNA approaches are permanent and irreversible and primarily target subcortical structures. These considerations have important implications for clinical trial design, execution, and patient selection, depending on the therapeutic approach.

A preponderance of the evidence presented in this review supports a positive risk-benefit profile for treatment with HTT-lowering ASO therapies that target either both *HTT* alleles or the variant *HTT* allele in adults with HD, and these approaches, as well as the AAV-mediated miRNA approach, are being tested in several ongoing clinical interventional studies that will begin to answer the pressing questions of how to best bring forward therapies to treat this disease.

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disease and similar disorders; receiving research funding from F. Hoffmann-La Roche, Teva Pharmaceutical Industries, and uniQure to perform basic research projects in mouse models of Huntington disease; and receiving personal compensation for administrative expenses as the coeditor-in-chief of the *Journal of Huntington's Disease*.

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