HUNTINGTON’S DISEASE

CPEB alteration and aberrant transcriptome-polyadenylation lead to a treatable SLC19A3 deficiency in Huntington’s disease

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Huntington’s disease (HD) is a hereditary neurodegenerative disorder of the basal ganglia for which disease-modifying treatments are not yet available. Although gene-silencing therapies are currently being tested, further molecular mechanisms must be explored to identify druggable targets for HD. Cytoplasmic polyadenylation element binding proteins 1 to 4 (CPEB1 to CPEB4) are RNA binding proteins that repress or activate translation of CPE-containing transcripts by shortening or elongating their poly(A) tail. Here, we found increased CPEB1 and decreased CPEB4 protein in the striatum of patients and mouse models with HD. This correlated with a reprogramming of polyadenylation in 17.3% of the transcriptome, markedly affecting neurodegeneration-associated genes including PSEN1, MAPT, SNCA, LRRK2, PINK1, DJ1, SOD1, TARDBP, FUS, and HTT and suggesting a new molecular mechanism in neurodegenerative disease etiology. We found decreased protein content of top deadenylated transcripts, including striatal atrophy-linked genes not previously related to HD, such as KTN1 and the easily druggable SLC19A3 (the ThTr2 thiamine transporter). Mutations in SLC19A3 cause biotin-thiamine–responsive basal ganglia disease (BTBGD), a striatal disorder that can be treated with a combination of biotin and thiamine. Similar to patients with BTBGD, patients with HD demonstrated decreased thiamine in the cerebrospinal fluid. Furthermore, patients and mice with HD showed decreased striatal concentrations of thiamine pyrophosphate (TPP), the metabolically active form of thiamine. High-dose biotin and thiamine treatment prevented TPP deficiency in HD mice and attenuated the radiological, neuropathological, and motor HD-like phenotypes, revealing an easily implementable therapy that might benefit patients with HD.

INTRODUCTION

Huntington’s disease (HD) is a devastating hereditary neurodegenerative disorder characterized by atrophy of the basal ganglia, particularly the striatum, and prominent motor symptoms (1). The underlying mutation is an expansion of a polyglutamine (polyQ)–encoding CAG repeat in the Huntingtin (HTT) gene (1), which is ubiquitously expressed, affecting additional brain areas beyond the striatum, as well as other parts of the body (2, 3). Although HTT-lowering strategies currently in clinical trials are promising therapeutic strategies (4, 5), their use may be limited by the mode of delivery to the affected brain areas and by side effect issues (5, 6). It is therefore important to continue investigating the molecular mechanisms by which the triggering mutation elicits toxicity to identify easily druggable targets.

Similar polyQ-encoding CAG mutations in different genes cause spinal-bulbar muscular atrophy, dentatorubral-pallidolysian ataxia, and multiple dominant spinocerebellar ataxias (SCAs) (7), and there is evidence of toxicity being mediated by both the expanded CAG-containing mRNAs and the polyQ-containing proteins (8, 9), the latter showing the propensity to self-aggregate (10, 11). One of the few genes able to act as a dual modifier of the toxicities induced by either CAG-repeat mRNA or polyQ in Drosophila models of SCA-3 is Orb2 (9), the ortholog of mammalian CPEB2-4.

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Cytoplasmic polyadenylation element binding proteins 1 to 4 (CPEB1 to CPEB4) are RNA binding proteins that recognize transcripts that harbor CPE sequences in their 3′ untranslated region (3′UTR), about 40% of the transcriptome (12, 13). CPEBs repress or activate their translation by inducing shortening or elongation of their polyadenine [poly(A)] tail (14). This CPEB-dependent regulation of transcriptome polyadenylation occurs in the cytoplasm and confers an additional layer of posttranscriptional regulation of gene expression (14, 15). CPEBs play a key role in early development (14), and they also act in adult neurons to enable synaptic plasticity through prion-like mechanisms (14, 16).

Altered CPEBs and subsequent alterations in transcriptome polyadenylation have been associated with various diseases such as cancer (17, 18), chronic liver disease (19), epilepsy (20), and autism (13), leading to the identification of new possible therapeutic targets among CPEB-dependent dysregulated genes. However, a potential role of CPEBs in neurodegenerative disorders has not been fully explored.

We noticed that HD-related genes are prevalent among genes that are mistranslated in the absence of CPEB1 (21). This, together with the known ability of CPEBs to modulate CAG/polyQ toxicity in flies (9), led us to characterize the status of CPEBs and of global mRNA polyadenylation in HD as a way to deepen our understanding of the molecular pathogenesis of HD and to explore new possible therapeutic targets.

RESULTS

Striatum of patients and mouse models with HD shows CPEB1/4 protein imbalance

To explore the status of CPEBs in HD, we performed Western blot analysis on postmortem striatal tissue from patients with HD and control subjects. This revealed markedly increased CPEB1 (303%, \( P = 6 \times 10^{-3} \)) and decreased CPEB4 (51%, \( P = 1.4 \times 10^{-5} \)) in the striatum of patients with HD (Fig. 1A), whereas no significant changes were observed regarding CPEB2 or CPEB3 (fig. S1A). We then explored whether a similar alteration of CPEBs takes place in mouse models of HD. We first analyzed the widely used R6/1 mouse model, which overexpresses exon1-mutant Htt, resulting in a robust, yet slowly progressing, motor phenotype. Similar to human samples, striatal homogenates from fully symptomatic R6/1 mice showed increased CPEB1 and decreased CPEB4 (Fig. 1B) without changes in CPEB2 or CPEB3 (fig. S1B). Next, we analyzed zQ175 mice, a heterozygous knock-in HD model with CAG expansion in the endogenous Htt gene that better resembles the human HD mutation but does not develop an overt motor phenotype within the maximal (about 2.5 years) life span of a mouse. In this model of premanifest HD, we only observed the decrease in striatal CPEB4 (fig. S1C). This prompted us to analyze presymptomatic and early symptomatic R6/1 mice. This revealed a tendency to decrease CPEB4 in 3-week-old R6/1 mice, which reaches significance (\( P = 4 \times 10^{-3} \)) in 6-week-old or older R6/1 mice, whereas the increase in CPEB1 protein content reaches significance (\( P = 1.4 \times 10^{-5} \)) at the age of 3 months (fig. S1D). These results demonstrate that, at least in R6/1 mice, CPEB4 decrease precedes CPEB1 increase.

To test whether the marked CPEB1/CPEB4 protein imbalance in symptomatic patients and mice with HD is due to matching changes in gene transcription, we performed real-time quantitative polymerase chain reaction (RT-qPCR) analysis (fig. S1, E and F). Regarding CPEB1, we observed a trend toward increased transcript expression in human HD striatum and a significant (\( P = 5 \times 10^{-3} \)) increase in R6/1 to controls or patients with HD and (B) wild-type (WT) or R6/1 mice striatum. (C) CPEB4 immunohistochemistry (\( n = 6 \) per genotype, 1 section per animal) and (D) CPEB4 (green) and HTT (red) immunofluorescence (\( n = 2 \) per genotype, 1 section per animal) in wild-type or R6/1 mice striatum. Examples of positive staining are indicated by arrows. CC, corpus callosum; CPu, caudate-putamen; LV, lateral ventricle. (A and B) Two-sided unpaired t test. *\( P < 0.05 \), **\( P < 0.01 \), and ***\( P < 0.001 \). Graphs show means ± SEM.
Altered transcriptome polyadenylation affects genes linked to major neurodegenerative diseases

We then tested whether the CPEB alteration observed in the striatum of patients and mice with HD correlates with changes in transcriptome polyadenylation. For this, we performed poly(U) chromatography followed by gene chip analysis of a pool of total RNAs from the striata of four R6/1 and wild-type mice. This revealed that R6/1 mice show increased transcript poly(A) tail length in 8.7% of the analyzed genes and decreased transcript poly(A) tail length in 8.6% (Fig. 2A and table S1). We performed gene ontology (GO) analysis [using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways] of the 1467 genes with absolute poly(A) fold change (FC) above 2 and found that the three terms with significant Benjamini-Hochberg corrected P values were as follows: HD (P = 3.3 × 10^−2), Alzheimer’s disease (AD; P = 4.3 × 10^−2), and Parkinson’s disease (PD; P = 4 × 10^−2) (Fig. 2B and table S2). This suggests that altered polyadenylation may contribute to the pathogenesis not only of HD but also across other common neurodegenerative disorders. We also performed Ingenuity Pathway Analysis in the genes showing shortening and lengthening of the poly(A) tail separately (fig. S2). Regarding diseases and disorders, the lowest P values were found for the genes with shortened poly(A) tails and corresponded to the terms “Neurological Disease” and “Developmental Disorder.” Regarding molecular and cellular functions, shortened-poly(A) genes again corresponded to the terms with the lowest P values, and these included “Cellular Assembly and Organization,” “Cellular Function and Maintenance,” “Cell Morphology,” and “Cell Death and Survival,” the latter also being found regarding lengthened-poly(A) genes. Genes playing key roles in neurodegeneration, such as those mutated in familial forms of AD/tauopathies, PD or amyotrophic lateral sclerosis (including PSEN1, MAPT, SNCA, LRRK2, PINK1, DJ1, SOD1, TARDBP, and FUS), and HD itself, showed altered polyadenylation (Fig. 2C and table S1), thus strengthening the notion that altered polyadenylation might play a role across the main neurodegenerative disorders. This may explain why some of the neurodegeneration-related genes, such as MAPT and GSK3β, have been reported to display detrimental altered protein expression in brains of patients with HD and mice without matching alterations in transcription (25, 26). Together, we have found that the striatum of symptomatic HD mice shows an alteration of poly(A) tail length in 17.3% of the transcriptome that markedly affects neurodegeneration associated genes, thus
suggesting a new molecular mechanism in the etiology of HD and possibly also in other major neurodegenerative diseases.

**Top deadenylated genes include striatal atrophy–linked genes and show decreased protein expression**

We analyzed the presence of CPE sequences in the UTR of the genes showing altered polyadenylation, and we observed an enrichment selectively in the genes showing deadenylation (Fig. 3A). Among the most markedly deadenylated genes (FC < −4.0), the percentage of CPE-containing genes was 93% (Fig. 3B). Shortening of the poly(A) tail is associated to diminished translation and decreased protein content (27), thus suggesting a possible CPEB-dependent decrease in protein expression of deadenylated transcripts. We confirmed reduced protein content of top deadenylated genes such as Autism susceptibility candidate 2 (AUTS2), Rho-associated coiled-coil containing protein kinase 1 (ROCK1), and Kinectin 1 (KTN1) both in HD and R6/1 striatal tissue, despite unaltered transcription (Fig. 3, B and C). Decreased KTN1 may be relevant to the striatal atrophy in HD because a genome-wide association study of common variants affecting volume of subcortical regions revealed that the size of the striatum is proportional to KTN1 gene expression (28). In addition, among the top deadenylated (FC < −4) genes was SLC19A3 (Fig. 3B) mutation of which causes biotin–thiamine–responsive basal ganglia disease (BTBGD; Online Mendelian Inheritance in Man (OMIM) #607483), a devastating neural disorder with prominent striatal involvement that can however be treated with a combination of the vitamins biotin and thiamine (29, 30).

**Patients with HD show a BTBGD-like thiamine deficiency**

SLC19A3 encodes the transmembrane thiamine transporter 2 (ThTr2), one of the transporters of thiamine (vitamin B1) (31). Individuals with BTBGD have decreased cerebrospinal fluid (CSF) thiamine content despite normal thiamine in blood (32, 33); bilateral atrophy in the head of the caudate nucleus and of the putamen; and a variety of neurological symptoms, including lethargy, irritability, dystonia,

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**Fig. 3.** Top deadenylated transcripts demonstrate decreased protein expression. (A) Incidence of CPEs in 3′UTR according to the mRNA poly(A) change. (B) Symbol, gene name, and number of CPEs of the most deadenylated transcripts in R6/1 mice. (C) AUTS2, ROCK1, and KTN1 protein and mRNA in the striata of controls or patients with HD and the striata of wild-type or R6/1 mice. (A) One-sided Fisher’s exact test. (C) Two-tailed unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001, and #P < 0.0001. Graphs show means ± SEM.
spasticity, tremor, and chorea, among others. All these symptoms improve upon administration of thiamine (34), to compensate for its decreased transport, and of biotin (vitamin B7) (34), which is believed to increase SLC19A3 transcription because individuals depleted of biotin show decreased SLC19A3 expression in peripheral blood cells (35, 36). In view of the observed marked deadenylation of SLC19A3 transcripts (Fig. 3A), we hypothesized that HD might, in part, phenocopy BTBGD due to a decrease in ThTr2 expression.

We next confirmed a marked ThTr2 decrease in both striatum and cortex of individuals with HD despite a tendency to increased transcript expression (Fig. 4A). This was mirrored by strongly decreased staining of the protein by immunohistochemistry in both striatum and cortex, which, in agreement with The Human Protein Atlas (proteinatlas.org), revealed neuronal and endothelial localization (Fig. 4B). In the CSF of individuals with HD, we observed decreased content of thiamine monophosphate (TMP), the prevailing form of thiamine in CSF, despite unaltered concentrations of thiamine in blood (Fig. 4C), resembling what has been reported for individuals with BTBGD (32, 33). In brain tissue, the predominant form of thiamine is thiamine pyrophosphate (TPP) (37), which is the bioactive form, acting as an enzyme cofactor for several mammalian enzymes in cellular metabolism (30). It is assumed that CSF thiamine deficiency in patients with BTBGD correlates with decreased brain content of the intracellular cofactor TPP, ultimately leading to neuronal dysfunction. To our knowledge, no data on brain TPP content are available regarding patients with BTBGD. However, we analyzed thiamine in postmortem HD striatum and found a marked decrease in TPP concentrations (Fig. 4D). Together, these results demonstrate a thiamine deficiency in HD brain and suggest that individuals with HD might benefit from thiamine and/or biotin supplementation therapy.

**HD mice show a BTBGD-like thiamine deficiency**

To preclinically test the potential of vitamin supplementation for HD, we first aimed to confirm that HD mouse models also show BTBGD-like features. Slc19a3 transcript expression in mice is essentially restricted to brain endothelium (vastdb.crg.eu), and we observed that ThTr2 protein was mostly absent from the brain vessels isolated from 5-week-old R6/1 mice (Fig. 5A) and its abundance was also reduced in brain vessels from 3-week-old R6/1 mice (Fig. S3A) and from 13-week-old zQ175 mice (Fig. 5B). We found, in R6/1 mice, indicators of thiamine deficiency similar to those observed in patients with BTBGD or in experimental rodent...
models of thiamine deficiency. For instance, the pyrithiamine-induced rat model of thiamine deficiency is characterized by decreased Glucose transporter 1 (GLUT1) in brain vessels and altered immunostaining with endothelial markers suggestive of vascular fragmentation (38), and we observed both alterations in R6/1 mice (Fig. 5, C and D). Similarly, patients with BTBGD show increased lactate and branched chain amino acids (32, 33), which are indicative of diminished activity of the TPP-dependent enzymes pyruvate dehydrogenase and branched chain α-keto acid dehydrogenase complex, respectively. Increased lactate has already been reported in brains of R6/1 mice (39), and regarding branched chain amino acids, we found increased isoleucine and leucine and a trend toward increased γ-valerolactone in the striata of R6/1 mice (Fig. S3B). Because other biochemical alterations reported in patients with BTBGD include increased content of certain organic acids such as 3-hydroxybutyric acid, glutaric acid, or 4-hydroxyphenyllactic acid (33), we analyzed these and other organic acids in the striata of R6/1 mice and detected increased 3-hydroxybutyric and glutaric acids and a trend toward increased 4-hydroxyphenyllactic acid (fig. S3C).

Next, we investigated whether the striata of HD mice showed decreased TPP and whether this reverts upon thiamine and biotin supplementation. We also tested whether, as postulated (35, 36), chronic administration of biotin at high dose results in increased SLC19A3 transcript. This was confirmed in the striata of 5-month-old R6/1 mice that received biotin (5 mg/kg per day) in the drinking water starting at the age of 3 weeks (fig. S3D). We also confirmed that, similar to patients with HD, R6/1 and zQ175 mice also showed decreased striatal concentration of TPP (Fig. 5, E and F). We also found that combined therapy of biotin and thiamine in the drinking water (B+T; see Materials and Methods) at doses similar to those used for patients with BTBGD (34), starting at the age of 3 weeks, when striatal CPEB4 decrease begins to be observed (fig. S1D) and the decrease of ThTr2 is already noticeable in vessels (fig. S3A), prevented the decreased striatal content of TPP in both R6/1 and zQ175 mice (Fig. 5, E and F).

**Fig. 5. Decreased ThTr2 and thiamine in HD mice.** (A and B) SLC19A3 protein (ThTr2) in brain vessels isolated from control and R6/1 mice (A) or control and zQ175 mice (B). (C) GLUT1 protein in brain vessels of control and R6/1 mice. (D) Rat Endothelial Cell Antigen-1 (RECA-1) and Endothelin 1 (ET-1) immunohistochemistry in the striata of control and R6/1 mice (n = 5 per genotype, 1 section per case). (E and F) Concentrations (nmol/g protein) of the different forms of thiamine in the striata of wild-type and R6/1 mice (E) or wild-type and zQ175 mice (F), with or without B+T. AC, anterior commissure. (A to C) Two-tailed unpaired t test. (E and F) Two-way ANOVA followed by Tukey’s post hoc test. *P < 0.05, **P < 0.01, and ***P < 0.001. Graphs show means ± SEM. n.s., not significant.

High B+T improves radiologic, neuropathologic, and motor phenotypes of HD mice
Then, we tested whether B+T treatment, which normalizes TPP in striatum of HD mice, was also able to improve any of their HD-like phenotypes. Although zQ175 mice do not show an overt motor phenotype, they do display striatal atrophy and phosphocreatine spectroscopy...
Fig. 6. Attenuation of HD-like phenotypes in mice upon treatment with biotin + thiamine (B+T). (A and B) In vivo T2-weighted MRI quantification of striatal volume of (A) untreated 17-week-old wild-type or zQ175 mice and (B) treated and untreated 24-week-old wild-type or zQ175 mice. (C) Analysis of phosphocreatine concentration by MR spectroscopy in the striata of wild-type or zQ175 mice with or without B+T, quantification on the left and example spectrum on the right. (D) Latency to fall off the rotarod for wild-type or R6/1 mice with or without B+T at 13 and 18 weeks (13 weeks: WT H2O, n = 4 male (m) and 10 female (f); WT B+T, n = 6 m and 12 f; R6/1 H2O, n = 6 m and 12 f; R6/1 B+T, n = 9 m and 16 f; 18 weeks: WT H2O, n = 6 m and 12 f; WT B+T, n = 6 m and 11 f; R6/1 H2O, n = 4 m and 9 f; R6/1 B+T, n = 5 m and 11 f). (E) Latency to fall off the inverted grid test for wild-type or R6/1 mice with or without B+T at 18 and 23 weeks (18 weeks: WT H2O, n = 3 m and 2 f; WT B+T, n = 4 m and 5 f; R6/1 H2O, n = 4 m and 5 f; R6/1 B+T, n = 5 m and 10 f; 23 weeks: WT H2O, n = 2 m and 6 f; WT B+T, n = 4 m and 10 f; R6/1 H2O, n = 3 m and 8 f; R6/1 B+T, n = 6 m and 11 f). (F) DARPP32-immunostained striatal (St) area in 19-week-old wild-type or R6/1 mice with or without B+T (WT H2O, n = 2 m and 1 f; WT B+T, n = 2 m and 1 f; R6/1 H2O, n = 3 m and 1 f; R6/1 B+T, n = 3 m and 1 f). (G) Quantification of cleaved caspase 3-positive cells in the brains of 19-week-old wild-type or R6/1 mice with or without B+T (WT H2O, n = 4 m and 2 f; WT B+T, n = 2 m and 1 f; R6/1 H2O, n = 4 m and 3 f; R6/1 B+T, n = 3 m and 1 f). (A) Two-tailed unpaired t test; (B to G) Two-way ANOVA followed by Tukey’s or Games-Howell post hoc test. *P < 0.05, **P < 0.01, and ***P < 0.001. Graphs show means ± SEM. ppm, parts per million.
CPEB4 overexpression attenuates R6/1 mouse HD-like phenotypes and ThTr2 deficit

To obtain mechanistic evidence that the observed pathogenic decrease of ThTr2 in R6/1 mouse is related to the described alterations in CPEB1 and CPEB4, we performed mouse genetics analysis, taking advantage of previously generated CPEB1-deficient [CPEB1 heterozygous knockout (KO), CPEB1−/−] (19) and CPEB4-overexpressing (CamKII-rtTA:CRE- CPEB4, TgCPEB4) (13) mice. These mice were bred with R6/1 mice to generate R6/1:CPEB1+/− and R6/1:TgCPEB4 mice. We first verified the attenuation of the CPEB1 increase and decrease seen in R6/1 mice with respect to wild type (Fig. 7, A and B). However, R6/1:CPEB1+/− mice performed similarly to R6/1 mice in the rotarod, the open field, and the inverted grid tests (Fig. S4, A to C). We then explored whether any of these attenuations affect the HD-like motor phenotype of R6/1 mice. We found that R6/1:TgCPEB4 mice did not demonstrate the motor coordination deficit and the hypoactivity observed in the rotarod and open field tests seen in R6/1 mice with respect to wild type (Fig. 7, A and B). However, R6/1:CPEB1−/− mice performed similarly to R6/1 mice in the rotarod, the open field, and the inverted grid tests (Fig. S4, C to E). The lack of positive effect of attenuating CPEB1 compared to the positive effect of restoring CPEB4 might, in part, be explained by the fact that the decrease of CPEB4 observed in R6/1 striatum (Fig. S1D) and in vessels isolated from R6/1 mice (Fig. S4F) precedes CPEB1 alterations, with CPEB4 changes coinciding with or preceding both the decrease of ThTr2 and the appearance of symptoms. In agreement with the ability of Orb2 (the drosophila ortholog of CPEB2 to CPEB4) to act as a modifier of CAG/polyQ toxicity in fly models (9), we found that the striatal atrophy and the number of apoptotic cells observed in R6/1 mice were attenuated in R6/1:TgCPEB4 mice (Fig. 7, C and D). Together, these results demonstrate a positive effect of attenuating the CPEB4 deficit of R6/1 mice, perhaps because this prevents the pathogenic abnormal transcript polyadenylation and altered protein expression of multiple genes, including SLC19A3. To further explore this, we took advantage of the available data on altered transcriptome polyadenylation in the cortex and striatum of CPEB4-deficient mice (13) and verified that depletion of CPEB4 resulted in decreased poly(A)-tail length, whereas CPEB4 overexpression resulted in increased poly(A)-tail length of the SLC19A3 transcript (Fig. 7E). Last, we confirmed that CPEB4 overexpression in R6/1:TgCPEB4 mice attenuated the decrease of ThTr2 observed in vessels isolated from R6/1 mice (Fig. 7F). Together, these results suggest that the decreased CPEB4 is pathogenic, at least in part, because it results in diminished polyadenylation of SLC19A3 mRNA and subsequent ThTr2 decrease.
The observed phenotypic improvement in HD mice upon CPEB4 overexpression suggests that alteration of CPEBs, especially the decrease in CPEB4, leads to the aberrant polyadenylation and subsequently altered protein expression of numerous etiology-relevant genes, such as SLC19A3. Theoretically, therapeutic strategies to correct the decrease in CPEB4 might lead to amendment of pathogenic gene misexpression beyond that of SLC19A3 and might therefore have additional positive effects with respect to those seen with B+T administration. However, pharmacological modulation of CPEB activity is challenging, particularly to counteract the decreased expression and/or activity seen in HD. A more efficient strategy to identify additional therapeutic targets from this study would be a systematic screening of all the neurodegeneration-associated misadenylated transcripts to see which ones might be druggable and then to verify altered protein content in patients with HD and mouse model tissue before preclinical testing, similar to what we have already done for SLC19A3.

There are some limitations of our study and its implications that deserve discussion. For instance, apart from motor symptoms, R6/1 mice also display some of the cognitive and psychiatric symptoms (55, 56) of patients with HD, and we have not explored whether these are also affected by the B+T therapy. In addition, given the importance of biotin and thiamine for the correct function of multiple mitochondrial enzymatic activities and the well-documented mitochondrial dysfunction in both HD (57) and BTBGD (33), a nonspecific attenuation of oxidative phosphorylation defects or structural mitochondrial abnormalities by the high-dose B+T therapy may also be contributing to observed beneficial effects in HD mice, beyond the identified SLC19A3-related deficits. In the absence of disease-associated mitochondrial deficits, high-dose vitamins might also boost some functional and morphological parameters, as evidenced by a trend to increased striatal volume in treated wild-type mice.

SLC19A3-unrelated aspects of the toxicity triggered by expanded CAG-repeat RNA and/or expanded polyQ will not be treated by B+T. There are also experimental therapeutic strategies based on preventing the toxic and early self-aggregation of polyQ (58) that can have a pleiotropic positive effect. Such anti-aggregation–related therapies could be combined with therapies aiming to correct particular alterations, such as the B+T treatment that we demonstrate here, because such combinations may result in synergistic effects.

In summary, this study reveals that alteration of CPEBs and of global mRNA polyadenylation emerges as a possible molecular mechanism in neurodegeneration. This study has pinpointed diminished ThTr2 as a pathogenic effector and revealed a brain thiamine deficiency in patients with HD, suggesting that vitamin supplementation regimes similar to those that benefit patients with BTBGD might be beneficial to individuals with HD.

**MATERIALS AND METHODS**

**Study design**

The objectives of this study were to (i) investigate the status of CPEBs in patients with HD and HD mice, (ii) analyze global mRNA polyadenylation in HD using the R6/1 mouse HD model, (iii) study the status of SLC19A3 and thiamine in patients with HD, and (iv) determine whether B+T treatment would alleviate HD pathology in HD mouse models. Sample size was determined by availability and previous experience with biochemical and behavioral characterization of the mouse models. A minimum of three individuals (human/mice) per group were used for studies involving statistical analyses, and the n for individual
experiments is indicated in the figures. Treated/nontreated mice (see the “Mouse biotin and thiamine treatments” section) were randomly allocated to experimental and control groups at weaning. Blinding was performed during data collection and analysis. Outliers were excluded using SPSS 26.0 (see statistical analysis). For all human studies, sampling was approved by the local ethics committee, and all subjects signed informed consent. For mouse studies, all experiments were performed according to the guidelines of the Animal Ethics Committee and were approved by the government authorities.

Human tissue samples

Human tissue samples used in immunoblot and immunohistochemistry were provided by the Institute of Neuropathology Brain Bank (HUBICO-IDIBELL, Hospital de Llobregat, Spain), the Neurological Tissue Bank of the IDIBAPS Biobank (Barcelona, Spain), the Banco de Tejidos Fundación CIEN (BT-CIEN, Madrid, Spain) and the Netherlands Brain Bank (Amsterdam, The Netherlands). Written informed consent for brain removal after death for diagnostic and research purposes was obtained from brain donors and/or next of kin. CSFs were collected in sterile tubes. Total blood was collected in K2E (EDTA) tubes (368801, BD Biosciences). CSF and blood were collected according to Hospital Universitario Ramón y Cajal (Madrid, Spain), Hospital Universitario Virgen del Rocío (Sevilla, Spain), and HUVR-IBIS Biobank (Andalusian Public Health System Biobank and ISCIII-Red de Biobancos PT13/0010/0056) guidelines. All the human samples were sex and age matched (table S3).

Animals

Different mouse models were used, which have been previously reported. Except for the R6/1 mice transgenic for the human exon-1-\(\text{Htt}\) gene (59) that were used in B6CBAF1 background, all other used mouse lines were used in pure B6 (C57BL/6) background: heterozygous knock-in of an expanded CAG track in exon 1 of \(\text{huntingtin}\) gene, zQ175 mice (40), HD94 mice with a tetracycline-conditional transgene encoding exon-1-\(\text{Htt}\) with an interrupted (CAG)\(_{94}\) repeat (60), CPEB1-deficient mice (CPEB1 heterozygous KO, CPEB1\(^{+/−}\)) (19), and CPEB4-overexpressing mice (CamkII-TTA.TRE-CPEB4, TgCPEB4) (13). When R6/1 mice are bred with any of the other genetically modified strains, all resulting genotypes present an equivalent mixed B6CBA genetic background (as a result of the B6CBAF1xB6 cross). In the resulting mixed background, the contribution of B6 is expected to be close to 75%, and the disparity from the expected background affects all experimental groups, including the wild-type controls, equally because they are all littermates. All mice were housed in Center for Molecular Biology “Severo Ochoa” (CBMOS) animal facility, with four per cage. Food and water were available ad libitum, and mice were maintained in a temperature-controlled environment on a 12-hour light/12-hour dark cycle with light onset at 08:00. Thiamine content in the chow diet was food pellets (7 mg/kg), according to the manufacturer (SAFE 150, from Safe-diets, France). Animal housing and maintenance protocols followed the local authority guidelines. Animal experiments were performed under protocols approved by the CBMOS Institutional Animal Care and Utilization Committee (Comité de Ética de Experimentación Animal del CBMOSO, CEEA-CBMSSO) and Comunidad de Madrid PROEX 293/15 and PROEX 247.1/20.

Mouse biotin and thiamine treatments

Mice were housed four per cage and given thiamine and/or biotin in the drinking water ad libitum. In a pilot group of mice, the volume of water intake was monitored per cage every 3 days; this revealed that each mouse drank, on average, 4 ml of water per day, regardless of whether water was supplemented with thiamine and/or biotin. To achieve the desired intake of vitamin/kg per day, the concentration of vitamins in the drinking water was calculated assuming that each mouse weighs 25 g. Controls for treatment and genotype were mice receiving plain water and nontransgenic littermates, respectively.

For experiments with R6/1 mice and their wild-type littermates, treatment began at 3 weeks of age, just after weaning. Biotin-only treatment consisted of 10 mg/kg per day; thiamine-only treatment started at a dose of 200 mg/kg per day, which was decreased to 50 mg/kg per day from 18 weeks, and combined biotin and thiamine (B+T) treatment consisted of biotin at 5 mg/kg per day and thiamine at 100 mg/kg per day, the latter being reduced to 25 mg/kg per day at the age of 18 weeks. The reason for decreasing thiamine doses from week 18 is that, in the pilot group of mice (for which the dose was not reduced), a possible toxicity in R6/1 mice was detected from 24 weeks of age, evidenced by an increase in volume drunk and excessive urination. These effects were not observed in the following groups for which thiamine concentrations were reduced to a quarter of the initial concentration. Biotin-only and thiamine-only treatments were analyzed in the pilot experiment, and no evidence of attenuation of the phenotype was observed in the motor coordination test (rotarod) or in the locomotor activity test (open field). For zQ175 mice and their wild-type littermates, B+T treatment consisted of biotin at 5 mg/kg per day with thiamine at 100 mg/kg per day starting at 5 or 18 weeks of age. For RT-qPCR analysis of Slc19a3 transcript, R6/1 and control mice were treated with biotin at 5 mg/kg per day, starting at the age of 4 weeks. The number of animals included in each group is indicated in Results and in the figures.

Statistical analysis

Statistical analysis was performed with SPSS 26.0 (SPSS Statistics IBM) and GraphPad Prism version 6.01. Data are represented as means ± SEM with 95% confidence interval. Outliers were plotted individually or not plotted; the criteria of exclusion were applied when point was further than 1.5*interquartile range away from the mean. The normality of the data was analyzed by the Shapiro-Wilk or Kolmogorov-Smirnov tests. Homogeneity of variance was analyzed by the Levene test. For comparison of two independent groups, two-tailed unpaired Student’s \(t\) test (data with normal distribution), Mann-Whitney-Wilcoxon, or Kolmogorov-Smirnov tests (non-normal distribution) were performed. To compare dependent measurements, we used a paired \(t\) test (normal distribution) or Wilcoxon signed-rank tests (non-normal distribution). For multiple comparisons, data with a normal distribution were analyzed by one- or two-way analysis of variance (ANOVA) followed by a Tukey’s or a Games-Howell’s post hoc test. Statistical significance of nonparametric data for multiple comparisons was determined by Kruskal-Wallis ANOVA. Enrichment tests were carried out with one-sided Fisher’s exact test. Life span was analyzed by log-rank (Mantel-Cox) test and represented with Kaplan-Meier plot. A cutoff value for significance of \(P < 0.05\) was used throughout the study.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs S1 to S4

Tables S1 to S3

Data files S1 and S2
REFERENCES AND NOTES

3. J. B. Carroll, G. P. Bates, J. Steffan, C. Saft, S. J. Tabrizi, Treating the whole body in Huntington’s disease. *Lancet* 14, 1135–1142 (2015).

7. H. T. Orr, H. Y. Zoghbi, Trinucleotide repeat disorders.

5. C. Sheridan, Questions swirl around failures of disease-modifying Huntington’s drugs.

16. K. Si, Y. B. Choi, E. White-Grindley, A. Majumdar, E. R. Kandel, Aplysia CPEB can form polyadenylation elements in Drosophila. *Nat. Struct. Mol. Biol.* 19, 577–585 (2012).

11. 3. J. B. Carroll, G. P. Bates, J. Steffan, C. Saft, S. J. Tabrizi, Treating the whole body in Huntington’s disease. *Lancet* 14, 1135–1142 (2015).

20. A. Parras, L. de Diego-Garcia, M. Alves, E. Beamer, G. Conte, E. M. Jimenez-Mateos, A. Ocampo, D. C. Henshall, R. Mendez, J. J. Lucas, Decreased glycogen synthase kinase-3 levels and activity contribute to Huntington’s disease. *Hum. Mol. Genet.* 20, 4810–4821 (2011).

14. M. Ivshina, P. Lasko, J. D. Richter, Cytoplasmic polyadenylation element binding proteins play a role in translational control. *Nature* 453, 1107–1111 (2008).

21. I. M. Alexandrov, M. Ivshina, D. Y. Jung, R. Friedline, H. J. Ko, M. Xu, B. O’Sullivan-Murphy, K. Si, S. Lindquist, E. R. Kandel, A neuronal isoform of the aplysia CPEB has prion-like properties. *Brain Sci.* 7, 13418 (2017).

22. K. Si, S. Lindquist, E. R. Kandel, A neuronal isoform of the aplysia CPEB has prion-like properties. *Brain Sci.* 7, 13418 (2017).

11. M. Pique, M. Fujimaki, P. Rogers, D. C. Rubinstein, Huntington-inhibiting strategies for Huntington’s disease. *Expert Opin. Investig. Drugs* 12, 1115–1132 (2003).

2. H. T. Orr, H. Y. Zoghbi, Trinucleotide repeat disorders. *Annu. Rev. Neurosci.* 30, 575–621 (2007).

1. F. O. Walker, Huntington’s disease. *Lancet* 369, 218–228 (2007).

14. A. Parras, H. Anta, M. Santos-Galindo, V. Swarup, A. Elorza, J. L. Nieto-Gonzalez, S. Pico, M. Pique, J. M. Lopez, S. Foissac, R. Guigo, R. Mendez, A combinatorial code for CPE-mediated translational control. *Science* 319, 575–621 (2008).

15. 3. J. B. Carroll, G. P. Bates, J. Steffan, C. Saft, S. J. Tabrizi, Treating the whole body in Huntington’s disease. *Lancet* 14, 1135–1142 (2015).

12.  M. Pique, M. Fujimaki, P. Rogers, D. C. Rubinstein, Huntington-inhibiting strategies for Huntington’s disease. *Expert Opin. Investig. Drugs* 12, 1115–1132 (2003).

2. H. T. Orr, H. Y. Zoghbi, Trinucleotide repeat disorders. *Annu. Rev. Neurosci.* 30, 575–621 (2007).

14. M. Ivshina, P. Lasko, J. D. Richter, Cytoplasmic polyadenylation element binding proteins play a role in translational control. *Nature* 453, 1107–1111 (2008).

21. I. M. Alexandrov, M. Ivshina, D. Y. Jung, R. Friedline, H. J. Ko, M. Xu, B. O’Sullivan-Murphy, K. Si, S. Lindquist, E. R. Kandel, A neuronal isoform of the aplysia CPEB has prion-like properties. *Brain Sci.* 7, 13418 (2017).

22. K. Si, S. Lindquist, E. R. Kandel, A neuronal isoform of the aplysia CPEB has prion-like properties. *Brain Sci.* 7, 13418 (2017).

2. H. T. Orr, H. Y. Zoghbi, Trinucleotide repeat disorders. *Annu. Rev. Neurosci.* 30, 575–621 (2007).

14. M. Ivshina, P. Lasko, J. D. Richter, Cytoplasmic polyadenylation element binding proteins play a role in translational control. *Nature* 453, 1107–1111 (2008).

21. I. M. Alexandrov, M. Ivshina, D. Y. Jung, R. Friedline, H. J. Ko, M. Xu, B. O’Sullivan-Murphy, K. Si, S. Lindquist, E. R. Kandel, A neuronal isoform of the aplysia CPEB has prion-like properties. *Brain Sci.* 7, 13418 (2017).

22. K. Si, S. Lindquist, E. R. Kandel, A neuronal isoform of the aplysia CPEB has prion-like properties. *Brain Sci.* 7, 13418 (2017).
T. I. Vlasova, S. L. Stratton, A. M. Wells, N. I. Mock, D. M. Mock, Biotin deficiency reduces expression of SLCl9A3, a potential biotin transporter, in leukocytes from human blood. *J. Nutr.** 135, 42–47 (2005).

T. B. Haack, D. Klee, T. M. Strom, E. Mayatepek, T. Meitinger, H. Prokisch, F. Distelmaier, Infantile Leigh-like syndrome caused by SLCl9A3 mutations is a treatable disease. *Brain** 137, e295 (2014).

L. Bettendorf, M. Peeters, P. Wins, E. Schoffeniels, Metabolism of thiamine triphosphate in rat brain: Correlation with chloride permeability. *J. Neurochem.** 60, 423–434 (1993).

S. Sarkar, S. Iachenko, M. G. Paule, J. Bowyer, J. P. Hanig, Brain endothelial dysfunction following pyrimidine induced thiamine deficiency in the rat. *Neurotoxicology** 37, 50–57 (2016).

T. T. Saing, B. Woodman, G. A. McLoughlin, J. L. Griffin, S. J. Tabrizi, G. P. Bates, E. Holmes, Metabolic characterization of the R6/2 transgenic mouse model of Huntington's disease by high-resolution MAS 1H NMR spectroscopy. *J. Proteome Res.** 5, 483–492 (2006).

T. Heikkinen, K. Lehtimaki, N. Vartiainen, J. Puivali, S. J. Hendricks, J. R. Glaser, A. Bradaia, K. Wadel, C. Touiller, O. Kontkanen, J. M. Yrjaneheiki, B. Buissin, D. Howland, V. Beaumont, I. Munoz-Sanjuan, L. C. Park, Characterization of neurophysiological and behavioral changes, MRI brain volumetry and 1H MRS in Q175 knock-in mouse model of Huntington's disease. *PLOS ONE** 7, e50717 (2012).

A. van Dellen, J. Welch, R. M. Dixon, P. Cordery, D. York, P. Styles, C. Blakemore, A. J. Hannan, N-Acetylaspartate and DARP-32 levels decrease in the corpus striatum of Huntington's disease mice. *Neuroreport** 11, 3751–3757 (2000).

A. M. Gown, M. C. Willingham, Improved detection of apoptotic cells in archival paraffin sections: Immunohistochemistry using antibodies to cleaved caspase 3. J. Histochim. Cytochem. 50, 449–454 (2002).

A. B. Byrne, P. Arts, S. W. Polyak, J. Feng, A. W. Schreiber, K. S. Kassahn, C. N. Hahn, D. A. Mordaunt, J. M. Fletcher, J. Lipsett, D. Bratkovic, G. W. Booker, N. J. Smith, H. S. Scott, Identification and targeted modulation of a neurodegenerative disorder caused by biallelic mutations in SLC5A6. *PNAS** 104, 28 (2019).

J. L. Rodrigo-Martin, N. Ozilbash, J. M. Lopez-Arrieta, Thiamine for Alzheimer's disease. *Cochrane Database Syst. Rev.** 2, CD001498 (2001).

G. E. Gibson, J. A. Hirsch, P. Fountzeli, B. D. Jordan, R. T. Cirio, J. Elder, Vitamin B1 (thiamine) and dementia. *Ann. NY. Acad. Sci.** 1367, 21–30 (2016).

G. E. Gibson, J. A. Luchsinger, R. Cirio, H. Chen, J. Franchino-Elder, J. A. Hirsch, L. Bettendorf, Z. Chen, S. A. Flowers, L. M. Gerber, T. Grandville, N. Schupf, H. Xu, Y. Stern, C. Habeck, B. Jordan, P. Fountzeli, Benfotiamine and cognitive decline in Alzheimer's disease: Results of a randomized placebo-controlled phase I clinical trial. *J. Alzheimers Dis.** 78, 989–1010 (2020).

R. Rodrigo-Melendez, J. Zempleni, Regulation of gene expression by biotin (review). *J. Nutr. Biochem.** 14, 680–690 (2003).

J. Motte, R. Gold, High-dose biotin in multiple sclerosis: The end of the road. *J. Neurosci. Res.** 98, 1957–1966 (2000).

B. G. Jenkins, H. D. Rosas, Y. C. Chen, T. Makabe, N. I. Mock, D. M. Mock, M. F. Beal, W. J. Koroshetz, 1H NMR spectroscopy studies of Huntington's disease: Correlations with CAG repeat numbers. *Neurology** 50, 1357–1365 (1998).

W. J. Koroshetz, B. G. Jenkins, B. R. Rosen, M. F. Beal, Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. *Ann. Neurol.** 41, 160–165 (1997).

H. D. Rosas, W. J. Koroshetz, B. G. Jenkins, Y. I. Chen, D. L. Hayden, M. F. Beal, M. E. Cudkowicz, Riluzole therapy in Huntington's disease (HD). *Mov. Disord.** 14, 326–330 (1999).

C. Y. Lin, Y. H. Hsu, M. H. Lin, T. H. Yang, H. M. Chen, Y. C. Chen, H. Y. Hsiao, C. C. Chen, Y. Chen, C. Chang, Neurovascular abnormalities in humans and mice with Huntington's disease. *Exp. Neurol.** 250, 20–30 (2013).

G. Kong, K. L. Cao, L. M. Judd, S. J. Renoir, A. J. Nannari, Microbiome profiling reveals gut dysbiosis in a transgenic mouse model of Huntington's disease. *Neurobiol. Dis.** 135, 104268 (2020).

C. L. Wassner, E. C. Mercieca, G. Kong, A. J. Nannari, S. J. McKewon, Y. Glikmann-Johnston, J. C. Stout, Gut dysbiosis in Huntington's disease: Associations among gut microbiota, cognitive performance and clinical outcomes. *Brain Commum. 2, fc0a110 (2020).

N. K. Mazarakis, A. C. Dybulks-Klosowicz, H. Grote, T. Pang, A. Van Dellen, M. Kossut, C. Blakemore, A. J. Hannan, Deficits in experience-dependent cortical plasticity and sensory-discrimination learning in presymptomatic Huntington's disease mice. *J. Neurosci.** 25, 3059–3066 (2005).

T. Y. Pang, X. Du, M. S. Zajac, M. L. Howard, A. J. Hannan, Altered serotonin receptor expression is associated with depression-related behavior in the R6/2 transgenic mouse model of Huntington's disease. *Hum. Mol. Genet.** 18, 753–766 (2009).

C. R. Carmo, L. Nafa, C. Lopes, A. C. Paj: Mitochondrial dysfunction in Huntington's disease. *Adv. Exp. Med. Biol.** 1049, 59–83 (2018).
interests. **Data and materials availability:** All data associated with this study are in the paper or the Supplementary Materials. Raw data of genome-wide analysis of mRNA polyadenylation in the striata of wild-type and R6/1 mice are available at GSE125544. The CPEB4-overexpressing mice and CPEB1-deficient mice can be made available to academic researchers under material transfer agreements by contacting J.J.L. (TgCPEB4) or R.M. (CPEB1 heterozygous KO). Human tissue specimens used in this research were obtained from the Institute of Neuropathology Brain Bank (HUBICO-IDIBELL, Hospital de Llobregat, Spain), the Neurological Tissue Bank of the IDIBAPS Biobank (Barcelona, Spain), the Banco de Tejidos Fundación CIEN (BT-CIEN; Madrid, Spain), Hospital Universitario Ramón y Cajal (Madrid, Spain), HUVR-IBIS Biobank (Andalusian Public Health System Biobank and ISCIII-Red de Biobancos PT13/0010/0056), and the Netherlands Brain Bank (Amsterdam, The Netherlands). Submitted 14 September 2020
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