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# Widespread alterations in microRNA biogenesis in human Huntington's disease putamen

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## Abstract

Altered microRNA (miRNA) expression is a common feature of Huntington's disease (HD) and could participate in disease onset and progression. However, little is known about the underlying causes of miRNA disruption in HD. We and others have previously shown that mutant Huntingtin binds to Ago2, a central component of miRNA biogenesis, and disrupts mature miRNA levels. In this study, we sought to determine if miRNA maturation per se was compromised in HD. Towards this end, we characterized major miRNA biogenesis pathway components and miRNA maturation products (pri-miRNA, pre-miRNA, and mature) in human HD (N = 41, Vonsattel grades HD2-4) and healthy control (N = 25) subjects. Notably, the striatum (putamen) and cortex (BA39) from the same individuals were analyzed in parallel. We show that Ago2, Drosha, and Dicer were strongly downregulated in human HD at the early stages of the disease. Using a panel of HD-related miRNAs (miR-10b, miR-196b, miR-132, miR-212, miR-127, miR-128), we uncovered various types of maturation defects in the HD brain, the most prominent occurring at the pre-miRNA to mature miRNA maturation step. Consistent with earlier findings, we provide evidence that alterations in autophagy could participate in miRNA maturation defects. Notably, most changes occurred in the striatum, which is more prone to HTT aggregation and neurodegeneration. Likewise, we observed no significant alterations in miRNA biogenesis in human HD cortex and blood, strengthening tissue-specific effects. Overall, these data provide important clues into the underlying mechanisms behind miRNA alterations in HD-susceptible tissues. Further investigations are now required to understand the biological, diagnostic, and therapeutic implications of miRNA/RNAi biogenesis defects in HD and related neurodegenerative disorders.

**Keywords:** Huntington's disease, microRNA, Ago2, Dicer, RNAi, Biogenesis, Autophagy

## Introduction

Huntington's disease (HD) is an incurable, hereditary neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in exon 1 of the Huntingtin (Htt) gene. At the protein level, this results in the generation of abnormal polyglutamine (PolyQ) repeats at the N-terminus of Htt. HD typically manifests itself in midlife with motor and cognitive symptoms associated with neurodegeneration in the striatum and, to a lesser degree, cortex. The molecular mechanisms leading to Htt-mediated neurodegeneration are still unresolved,

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although it is well recognized that abnormal regulation of gene expression is an early and critical feature of HD neuropathology [26, 35, 38].

The small non-coding microRNAs (miRNAs) play a central role in gene expression regulation by promoting messenger RNA (mRNA) translation inhibition and/or degradation [48, 49]. MiRNA function is inherently related to its maturation that follows two major processing steps [15, 32]. First, the long primary miRNA transcript (pri-miRNA) is cleaved by the Drosha/DGCR8 complex to generate a ~70 nucleotide (nt) precursor miRNA (pre-miRNA). The pre-miRNA is transported to the cytoplasm by Exportin 5, where it is then cleaved by Dicer to generate a ~22 nt mature miRNA. The mature miRNA is finally loaded with Ago2 and associated proteins (e.g., TRBP) into the endogenous RNA-induced silencing complex (RISC) that binds to the 3'untranslated region (3'UTR) of target mRNAs. Interestingly, miRNAs can control diverse biological pathways by modulating one or several key target genes simultaneously [43]. Therefore, any disruption in this pathway could have deleterious consequences on gene expression networks and cell homeostasis.

Indeed, it is now well established that miRNAs are essential to the survival of striatal and cortical neurons [5, 16]. Loss of neuronal Dicer in adult mice leads to alterations in transcription, reduced brain size, behavioural defects, and decreased lifespan [6, 9], reminiscent of some HD features. In HD mice (YAC128 model), Lee et al. observed a global upregulation or downregulation of mature miRNAs in the early and late stages of the disease, respectively [28]. These changes coincided with transient changes in Dicer, Drosha and Exportin mRNA levels. Recently, we and others have shown that mHtt binds to Ago2 protein [34, 41, 42], whereas transient overexpression of mHtt in cells and mice leads to higher Ago2 expression and widespread alterations in mature miRNA levels [34]. Furthermore, post-mortem studies have detected changes in mature miRNA expression and editing profiles in the brains of HD mice and humans [13, 20–22, 27, 29, 30, 33].

Despite these observations, there is surprisingly no clear evidence that miRNA maturation per se is defective in HD, especially in humans. This could have important implications for understanding miRNA regulation and function within cell survival pathways and current therapeutic efforts using the endogenous RISC (composed of Ago2 and Dicer) to silence mHtt [1, 8]. Towards this end, we have analyzed, for the first time, all major miRNA pathway components and maturation products (pri-miRNA, pre-miRNA, mature) in human HD tissue samples. Notably, our experiments were conducted in different tissues collected from patients at various stages of the disease. In sum, our data implicate widespread defects in the pre-miRNA to mature miRNA step in HD, which overlaps with mHtt pathology and overt neurodegeneration in the striatum.

## Materials and methods

### Human brain samples

Dissected frozen human putamen and matching cortical (BA39 region) tissues (0.5–1.2 g per sample) were obtained from the Harvard Brain Tissues Resource Center via NIH Neurobiobank (Table 1) as before [47]. This specific study included brain tissues from 25 control and 41 HD individuals. Frozen post-mortem tissues were prepared as described previously and used for protein and RNA analysis [47]. CAG-repeat length was determined by the CHU de Québec Sequencing and Genotyping platform using a 6-FAM fluorescent primer (Applied Biosystems Inc, Foster City, CA, USA) in a polymerase chain reaction (PCR), followed by the determination of the product size by capillary electrophoresis in a 3130xl Genetic analyzers. We used the disease burden score (DBS) to estimate the lifetime exposure to mutant huntingtin in individuals with HD with the following equation:  $DBS = \text{age at death} \times (\text{CAG-repeat length} - 35.5)$ .

### Protein and RNA extraction

Total proteins were extracted as previously described [47]. In brief, frozen tissues were mechanically homogenized in seven volumes of lysis buffer (150 nM NaCl,

**Table 1** Characteristics of the individuals providing post-mortem brain samples (NIH NeuroBioBank)

|         | N  | Age        | PMI        | Women (%) | Men (%) | CAG Allele 1 | CAG Allele 2 | Disease score |
|---------|----|------------|------------|-----------|---------|--------------|--------------|---------------|
| Control | 25 | 67 [35–79] | 20 [8–24]  | 40        | 60      | 21           | 24           | –             |
| HD2     | 10 | 64 [49–80] | 21 [12–27] | 60        | 40      | 22           | 47           | 703           |
| HD3     | 23 | 59 [47–75] | 20 [8–27]  | 39        | 61      | 25           | 48           | 726           |
| HD4     | 8  | 52 [47–64] | 19 [12–24] | 50        | 50      | 23           | 52           | 844           |
| HD      | 41 | 59 [43–75] | 20 [12–27] | 46        | 54      | 24           | 49           | 758           |

50 mM Tris, 0.5% deoxycholate, 1% Triton X-100, 0.5% sodium dodecyl sulfate (SDS), cOmplete™ protease inhibitor cocktail (Roche), 1 mM of sodium fluoride and 1 mM of activated orthovanadate as phosphatase inhibitor), then sonicated three times for 5 X 1-s pulses. The solution was spun at 100,000 g for 20 min at 4 °C. The supernatant (soluble proteins) was kept at –80 °C until processed. The pellet was further homogenized in formic acid (FA) and spun for 20 min at 17,500 g at 4 °C. FA-soluble proteins (FA fraction) were dried before being sonicated in NuPAGE® LDS sample buffer (Life Technologies) supplemented with 0.1 M of dithiothreitol, incubated 10 min at 70 °C and kept at –80 °C until processed. Soluble proteins were quantified with Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific) and mixed to the NuPAGE® LDS sample buffer with 5% final volume of β-mercaptoethanol, then boiled 10 min at 95 °C for Western blot analysis. Total RNA was extracted from frozen tissues using TRIzol reagent (Ambion by Life Technologies) according to the manufacturer's instructions. Total RNA pellet was suspended in RNase free water, quantified, and diluted to a final concentration of 100 ng/μL. RNA was kept at –80 °C until processed for qRT-PCR analysis.

#### Western blotting

Five to twenty micrograms of soluble proteins were separated by two different systems: 10% SDS–polyacrylamide gels (SDS–Page) and gradient 3–15% tris–acetate polyacrylamide gels for higher and lower molecular weight proteins. For the 10% SDS–Page, proteins were transferred onto a 0.45 μm nitrocellulose membrane (Bio-Rad, catalogue no 1620115) for 1 h at RT at 100 V. For the gradient gels, proteins were transferred onto a 0.45 μm methanol-activated PVDF membrane (Immobilon, Millipore) overnight at 4 °C at 25 V and 45 min at 4 °C at 75 V the next day. The membrane was blocked with 5% non-fat milk and 1% bovine serum albumin, then incubated overnight at 4 °C with the appropriate primary antibodies (see Additional file 2: Table S1). On the second day, membranes were incubated with appropriate secondary anti-IgG–HRP antibodies (Jackson ImmunoResearch: anti-mouse, catalogue no 115-035-146 or anti-rabbit, catalogue no 111-035-144) at RT for 1 h. The immune-reactive bands were acquired using Immobilon Western Chemiluminescent HRP Substrate (Millipore) and visualized with the Fusion FX (Vilber Lourmat, Eberhardzell, Germany) imaging system. Normalization

was performed on total proteins obtained with Ponceau Red or StainFree staining. Band intensities were quantified using the ImageJ software.

#### Dot blot

Two microliters of each sample were slowly spotted on the nitrocellulose membrane. After drying the membrane, non-specific sites were blocked, and the membrane was processed as described in the Western Blotting section. Dot intensity was normalized on the total amount of tissue used for the extraction.

#### Primary microRNA real time quantitative RT-PCR

The reverse transcription was performed with 500 ng of total RNA using the High-capacity cDNA reverse transcription kit (ThermoFisher Scientific, catalog no 4368814) according to the manufacturer's instructions. Program: 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 min. cDNA was stored at –20 °C until further processing. The real-time quantitative PCR (qRT-PCR) was performed with TaqMan Fast Advanced Master mix (ThermoFisher Scientific, catalog no 4444963) according to manufacturer's instructions. Primers were purchased from ThermoFisher Scientific (Hs03302879\_pri, hsa-mir-10b; Hs03303255\_pri, hsa-mir-127; Hs03303101\_pri, hsa-mir-128-1; Hs03303111\_pri, hsa-mir-132; Hs03293754\_pri, hsa-mir-196b; Hs03302957\_pri, hsa-mir-212). Primary microRNAs were normalized to the geographic mean of GAPDH and RPL32. The relative amount of each primary microRNA was calculated using the comparative Ct ( $2^{-\Delta\Delta C_t}$ ) method as before [46].

#### Precursor microRNA real time quantitative RT-PCR

The reverse transcription was performed with 500 ng of total RNA using the miScript RT II kit (Qiagen) according to the manufacturer's instructions. The RT-PCR was performed with the Hiflex buffer, as recommended by the manufacturer to study precursor microRNAs. Program: 38 °C for 60 min and 95 °C for 5 min. cDNA was stored at –20 °C until further processing. The qRT-PCR was performed with QuantiTect SYBR Green PCR Master Mix (Qiagen) according to the manufacturer's instructions. miScript precursor assay primers were purchased from Qiagen (mir-10b ID: MP00003983; mir-127-1 ID: MP00004123; mir-128-1 ID: MP00004137; mir-132 ID: MP00004179; mir-196b ID: MP00004935; mir-212 ID: MP00004256). Precursor microRNAs were normalized to SNORD95 (ID: MS00033726). The relative amount of each precursor microRNA was calculated using the comparative Ct ( $2^{-\Delta\Delta C_t}$ ) method.

### Mature microRNA real time quantitative RT-PCR

The reverse transcription was performed with 10 ng of total RNA using the TaqMan MicroRNA Reverse transcription kit (ThermoFisher) according to the manufacturer's instructions. Program: 16 °C for 30 min, 42 °C for 30 min and 85 °C for 5 min. cDNA was stored at -20 °C until further processing. The qRT-PCR was performed with TaqMan Fast Advanced Master mix (ThermoFisher Scientific) according to the manufacturer's instructions. miRNA assay primers were purchased from ThermoFisher Scientific (hsa-miR10b, 002218; hsa-miR127, 000452; hsa-miR128, 002216; hsa-miR132, 000457; hsa-miR196b, 002215; hsa-miR212, 000515). Mature microRNAs were normalized to the geographic mean of RNU48 (hsa-RNU48, 001006) and Let-7f (hsa-Let-7f, 000382). The relative amount of each mature microRNA was calculated using the comparative Ct ( $2^{-\Delta\Delta C_t}$ ) method.

### Statistical analysis

All graphics and statistical analyses were performed using GraphPad Prism 9 Software (Graph Pad Software, Inc., La Jolla, CA, USA). Normality and lognormality tests were performed, and parametric or non-parametric tests were used accordingly. When sample distribution passed the normality test, a parametric one-way analysis of variance (ANOVA) test followed by Dunnett's multiple comparisons and parametric unpaired student t-test were performed. When sample distribution did not pass the normality test, a non-parametric Kruskal–Wallis test followed by Dunn's multiple comparisons and a non-parametric Mann Whitney student t-test were performed. The threshold for statistical significance was set to  $p$ -values < 0.05.

## Results

### Comparative biochemical analysis of Htt pathology in cortex and striatum

To understand the impact of endogenous human Htt on miRNA maturation, we first evaluated Htt expression and pathology in two different brain regions in human HD (Table 1). We quantified the amount of Htt protein in 41 HD patients (N=10 HD2, N=23 HD3, N=8 HD4) and 25 Controls from matching striatal and cortical tissues. Consistent with earlier findings, we observed a decrease in soluble total (full-length) Htt in the HD striatum using 1HU-4C8 and CH00146 antibodies (Fig. 1A–E). However, no significant changes in total Htt were shown in the HD cortex. Using the 1HU-4C8 clone, we detected an increase in N-terminal fragments (MW ~ 40–50 KDa) in both brain regions. As expected, an increase in formic acid (FA)-insoluble aggregated mHtt was also observed

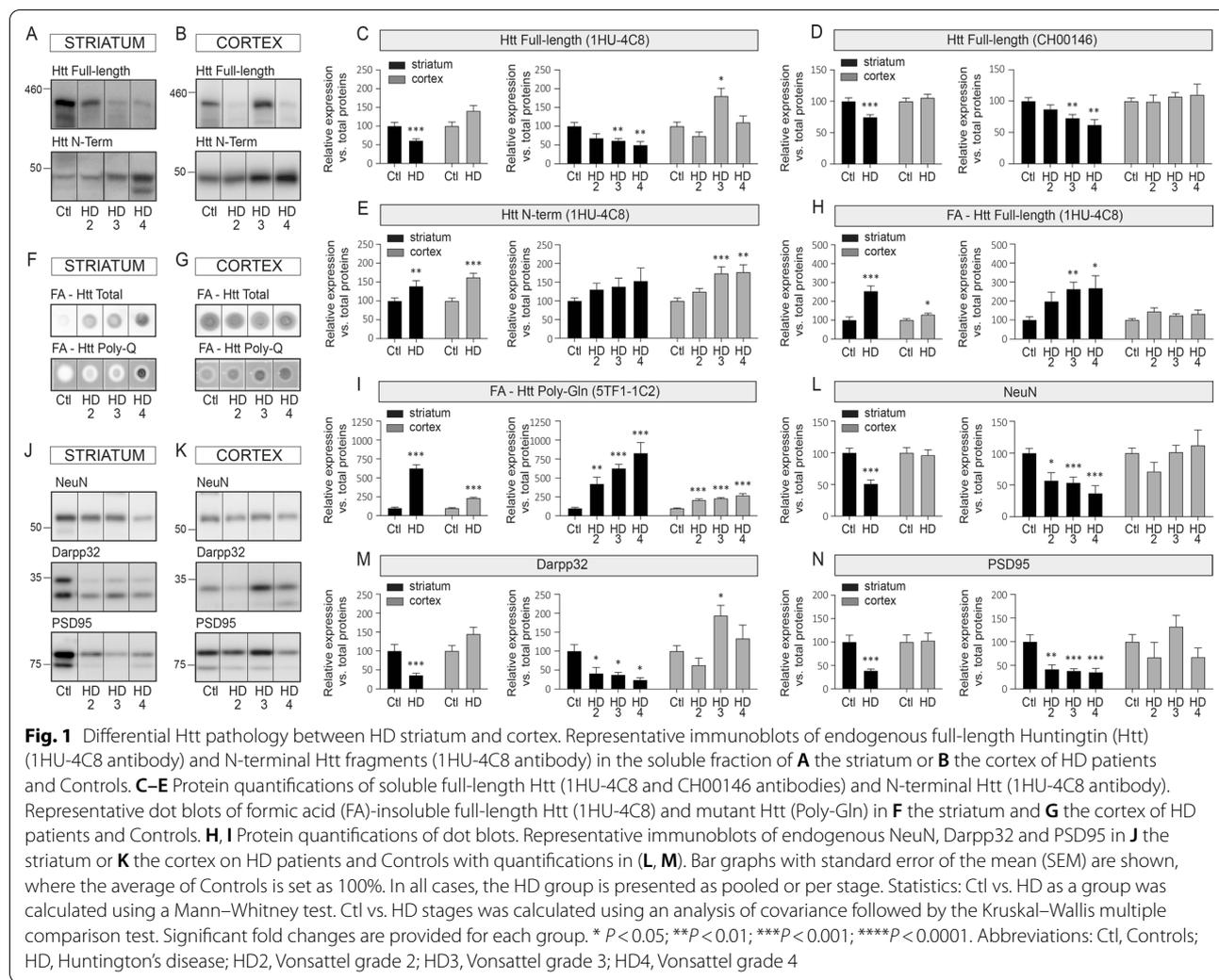
in both regions using an anti-PolyQ antibody, although higher levels were apparent in the striatum (Fig. 1H, I). Along with these results, significant decreases in NeuN (neuronal marker), DARPP-32 (striatal neuron marker), and PSD-95 (postsynaptic marker) protein levels were observed in the HD striatum while the cortex was mostly spared (Fig. 1L–N). Our results support that the relative expression level of these proteins is modulated between brain regions (Additional file 3: Fig. S1). These data suggest that Htt aggregation is not the consequence of an increased expression level of endogenous Htt and cannot be directly correlated with neuronal loss. Thus overall, in line with previous results suggesting that HD pathology starts in the striatum, the striatal tissue samples analyzed herein presented severe signs of Htt pathology and neurodegeneration compared to the parietal cortex of the same individuals.

### Early-stage alterations of miRNA pathway components in human HD striatum

Previous studies in mice [28, 34] showed that specific members of the miRNA biogenesis pathway are compromised in HD models. In human brains, we observed a robust decrease in Drosha, Dicer, and Ago2 protein levels from HD2 in the striatum but not in the cortex (Fig. 2A–H). No significant changes in Dicer mRNA were noted in either region (Additional file 3: Fig. S2), suggesting that alterations in expression occurred at the post-transcriptional level. Modest or transient variations in DGCR8 and TRBP proteins were seen in these samples with no changes in Exportin 5. Taken together, these results suggest that core miRNA biogenesis pathway components are rapidly and specifically compromised in the human HD striatum and precede overt neurodegeneration.

### miRNA expression analysis in HD brain

Having shown that several major miRNA biogenesis components were compromised in the human HD brain, we next aimed to determine potential effects on miRNA levels. We performed a literature search to identify HD-related miRNAs for downstream functional analyses. Following an initial screen of 16 candidates previously associated with HD, we selected a panel of six conserved miRNAs that were commonly misregulated in both HD striatum and cortex (Fig. 3 and Additional file 3: Fig. S3). These included miR-10b, miR-196b, miR-127, miR-128, miR-132 and miR-212. To avoid bias, we chose up and downregulated miRNAs in HD. The different genomic sources of miRNAs further influenced our final choice: miR-10b and miR-196b are generated from introns of host coding genes, miR-132 and miR-212 are



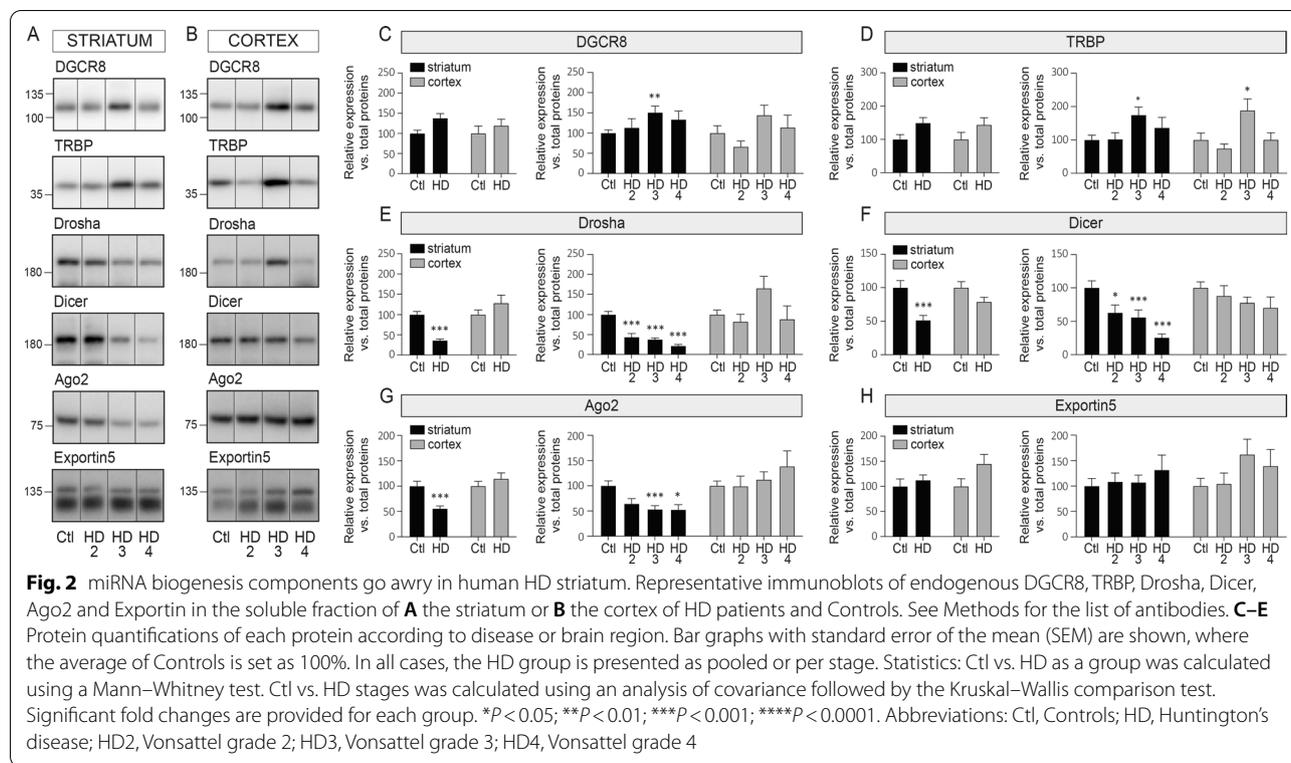
co-expressed as a cluster from the same non-coding gene, miR-127 is expressed from a much larger non-coding miRNA cluster, whereas miR-128 is transcribed from an individual intergenic non-coding gene.

We quantified all three types of miRNA maturation products (primary, precursor, mature) in the human striatum (Fig. 3A) and cortex (Fig. 3B) using a distinct set of normalization genes (Additional file 3: Fig. S4). As expected, we observed a co-expression of intronic miRNAs and host genes in HD (i.e., miR-10b and miR-196b in the striatum), as documented before [20] (Additional file 3: Fig. S2). Surprisingly, however, various other types of phenomena were observed outside of this canonical pattern, some of which were tissue and disease-stage specific. One example includes the downregulation of pri-miR-127 and mature miR-127, but not pre-miR-127, in the HD striatum. Another example consists of the specific downregulation of mature miR-132 in HD striatum but an overall downregulation of pri-miR-132,

pre-miR-132 and mature miR-132 in HD cortex. In sum, these results suggest that miRNA maturation is controlled at both transcriptional and post-transcriptional levels in the HD brain.

### Prominent pre-miRNA to mature miRNA maturation deficits in HD

To better grasp any changes in miRNA maturation per se in HD, we analyzed overall ratios (inhibition scores) between a given miRNA precursor and its substrate, as initially proposed by Emde et al. [10]. Despite rare exceptions, the inhibition scores between pri-miRNA and pre-mRNA were essentially normal in the HD striatum and cortex (Fig. 3C). On the other hand, the inhibition scores between pre-mRNA and mature miRNA were drastically altered for all tested miRNAs in the HD striatum (Fig. 3D). In contrast, only miR-10b and miR-196b reached significance in the late-stage HD cortex. Interestingly, miRNA levels and inhibition



scores were unaffected in human HD blood in a separate cohort (Additional file 3: Fig. S5). Taken together, these results implicate early and robust deficits in the pre-miRNA to mature miRNA maturation step in the human HD striatum.

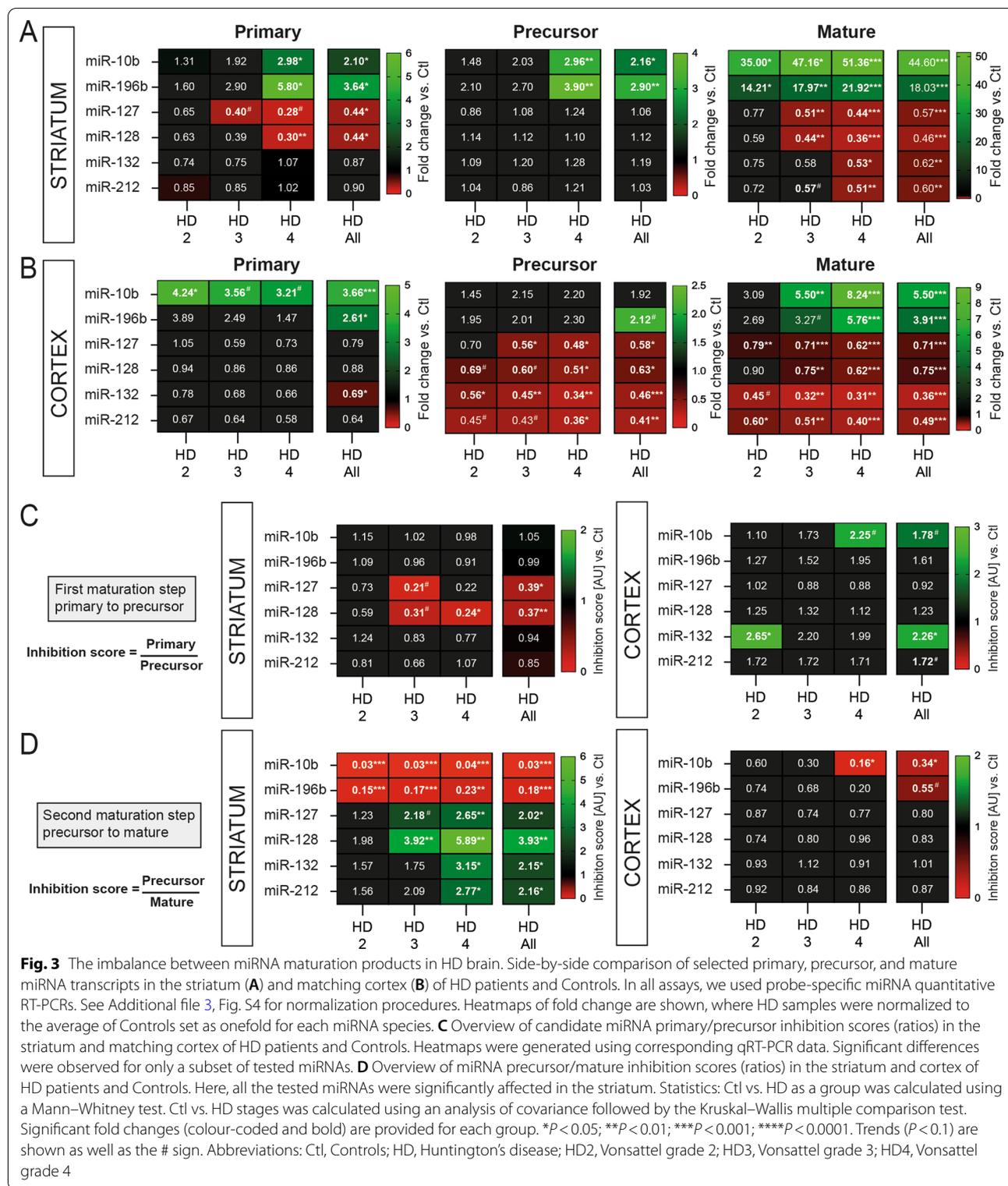
**Autophagy dysfunction overlaps with miRNA maturation defects in HD**

Finally, we set out to better understand the molecular mechanisms responsible for miRNA maturation defects in HD. Autophagy dysfunction is an inherent feature of HD, and we have previously shown that it influences mature miRNA levels in vivo [34]. Accordingly, we observed a strong and significant downregulation of major markers of autophagy, namely P62, LC3 and Beclin, in human HD striatum but not cortex at all stages of the disease (Fig. 4A–G). A detailed analysis of LC3-I and LC3-II using a 20% acrylamide gel revealed a non-significant trend for higher LC3-II in HD3 and HD4 stages in the putamen (data not shown). Interestingly, TDP-43 levels, previously implicated in regulating miRNA maturation in vitro [3, 23], did not correlate with miRNA defects (Fig. 4A, B, H). Taken together, these results strengthen the role of autophagy in modulating miRNA maturation in HD-susceptible brain regions.

**Discussion**

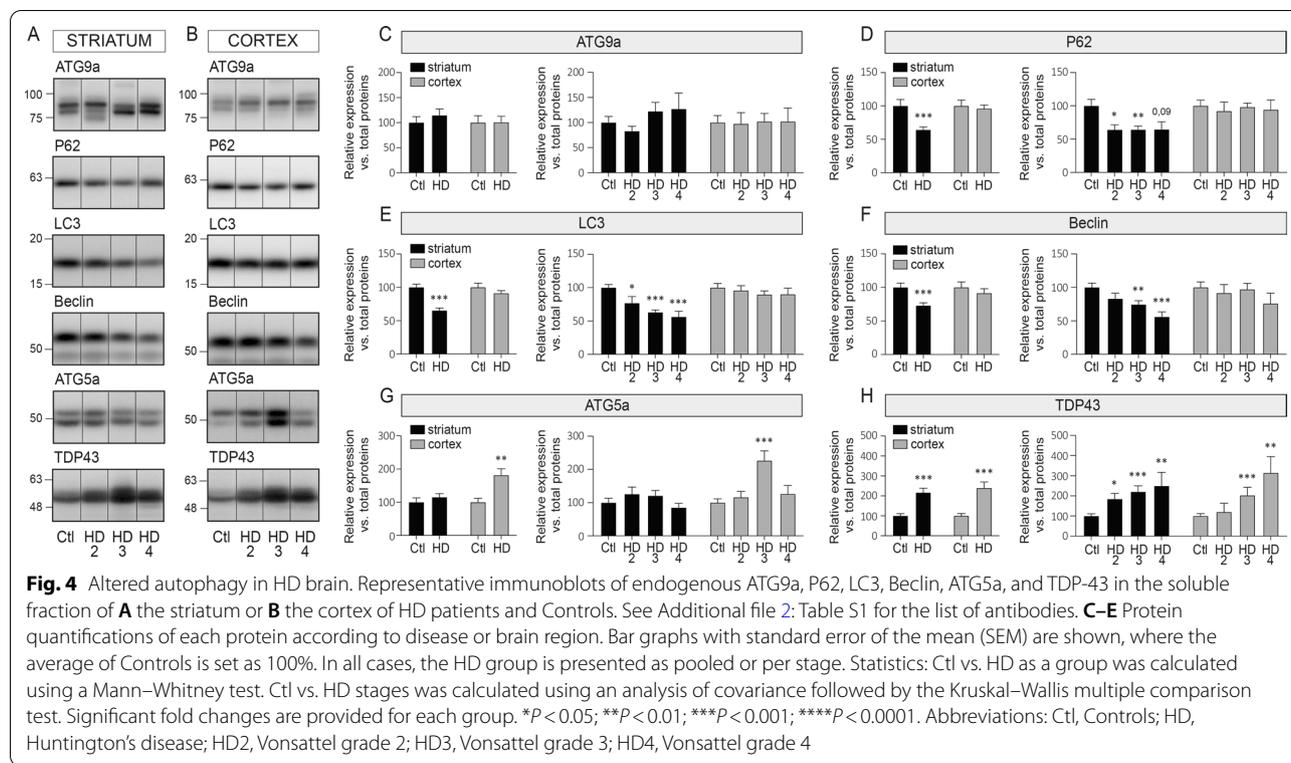
This study provides the first in vivo evidence that miRNA maturation is dysregulated in human HD and sheds new light on the causes and potential implications of miRNA dysregulation in HD. The importance of our findings is severalfold: (1) they provide important clues on intra-individual variability and susceptibility towards mHtt pathology, (2) they could explain a substantial proportion of miRNA alterations previously documented in HD brain, (3) they provide a first in-depth analysis of the RISC components necessary for endogenous RNA interference (RNAi), (4) they support the potential importance of specific miRNAs (and downstream targets) in HD pathogenesis, and finally, (5) they strengthen the broad implications of autophagy dysregulation in HD pathogenesis.

To our knowledge, this is the first characterization of human Htt expression and aggregation in two different brain regions in HD from the same individuals. These experiments validate and extend our previous biochemical studies on Htt pathology and other proteinopathies exclusively in the striatum (putamen) [47]. In agreement with earlier reports, lower Htt (mHtt) expression (loss-of-function) and higher mHtt aggregation (gain-of-function) are likely both contributing factors to the severe



neurodegeneration observed in the striatum, although other biological factors might also come into play (see also below). Interestingly, (at least some of) the proposed

toxic Htt N-terminal fragments [36, 51] were upregulated in both brain regions analyzed, further suggesting that additional factors participate in Htt-mediated toxicity.



This hypothesis is consistent with the role of miRNA-dependent survival pathways in this process.

Remarkably, very little is known about the underlying causes of miRNA alterations in HD, which is key to understanding the role, impact, diagnostic, and therapeutic potential of miRNAs in human brain diseases. In this regard, the BA39 cortical region is of interest because excluding neuronal cell death as a direct or indirect cause of miRNA changes. The inhibitory effects of mHtt on transcription [26, 35, 38] are readily evident in this study on both coding (e.g., miR-10b) and non-coding (e.g., miR-127) genes and their host miRNA transcripts. In addition to transcriptional effects, our observations implicate other molecular mechanisms as significant causes of mature miRNA disruption in HD. Identifying factors that control pre-miRNA to miRNA maturation abnormalities in HD (e.g., transport, cleavage, sequestration, degradation) will require further investigation.

Interestingly, cellular stress has been shown to disrupt pre-miRNA to mature miRNA genesis in ALS [10]. Stress can influence miRNA maturation in several ways, including the sequestration of pre-miRNAs and pathway components (e.g., Ago2) to P bodies and/or stress granules. In this line of thought, autophagy is functionally implicated in mHtt protein turnover and aggregation and, more recently, miRNA maturation [2, 50]. More studies are

required to understand the cause-and-effect relationship between these factors during HD progression.

Interestingly, most miRNA biogenesis components were downregulated in the human HD striatum. This observation is somewhat consistent with earlier findings in mice that showed a transient shift (up to down) in miRNA expression levels during disease progression. It remains to be elucidated whether the triggering factor is a unique component (e.g., Ago2 downregulation [28]) or a more general mechanism in humans. Also, it is uncertain if changes in RISC components could explain miRNA editing patterns in HD [13]. The study of pre-symptomatic HD patients (i.e., Vonsattel grades HD0-1) or humanized cell models (e.g., iPSC) would help address these questions. In any case, our results are consistent with abnormal regulation of miRNA biogenesis in HD.

We did not observe changes in mature miRNA levels (not shown) or miRNA maturation defects in HD blood. We and others have previously reported high expression levels of Htt in blood cells [7, 37], further strengthening the hypothesis of tissue-specific effects. However, we cannot exclude maturation defects for other miRNAs and/or cohorts at this stage. A critical question is how mature miRNAs become dysregulated in tissues or cell types with seemingly normal miRNA biogenesis. As shown herein, changes in gene transcription can lead

to alternations of miRNA host genes and, henceforth, mature miRNA output. In addition, and as mentioned above, mature miRNA levels are subjected to multiple regulatory mechanisms (e.g., degradation) and feedback loops that can go awry in disease conditions. An attractive hypothesis is that the specific disruption of miRNA biogenesis—and not the indirect effects of neurodegeneration on mature miRNA levels—is responsible for the early susceptibility of cell loss in HD. This could have context-specific consequences on key miRNAs or other RISC-dependent RNAs required to maintain cell homeostasis.

In this line of thought, several groups have already tested the regulatory effects of candidate miRNAs on HD pathology, behaviour, and cell survival. For example, an increased expression of miR-196a (homologue of miR-196b) in transgenic mice caused lower mHtt expression and aggregation in an HD model [4]. Overexpression of miR-10b in PC12 cells expressing mHtt also increased cell survival [20]. Finally, the brain supplementation of miR-132 in HD mice partially rescued behavioural and motor symptoms [11]. Interestingly, the miR-132/212 cluster is among the most strongly affected miRNA (family) in the HD brain (this study and [11, 27]). We have previously shown that miR-132/212 knockout mice display autophagy abnormalities and lower BDNF levels in the brain, as seen in HD [17, 18, 40, 52]. Additional studies are required to establish the underlying causes of Drosha, Dicer and Ago2 downregulation in HD striatum, although autophagy is a reasonable candidate. The challenge now is to identify the targets and pathways regulated by mature and possibly immature miRNA transcripts for in-depth functional analyses *in vivo*, considering the occurrence of potential transient changes as observed in HD mice and tissue-specific effects.

Interestingly, recent evidence suggests that impaired miRNA maturation occurs in other trinucleotide repeat disorders. For instance, the expanded CGG repeats in FMRP (causing Fragile X-associated tremor/ataxia syndrome) sequester DGCR8 and Drosha and disrupt miRNA maturation in mice [44]. In *Drosophila*, mutant ataxin-2 (causing spinocerebellar ataxia type 2) disrupts Ago expression and miRNA function [31]. MiRNA maturation is also impaired in models of expanded polyQ within ataxin-3 (causing Machado-Joseph disease), whereas blocking miRNA biogenesis increased ataxin-3 aggregation [24]. These observations strongly suggest that miRNA alterations in these disorders are a direct consequence of disease genes (e.g., sequestration) rather than an indirect effect of cell stress or other factors. The fact that Htt binds to Ago2 is consistent with this hypothesis, although a role for additional genetic or molecular mechanisms cannot be excluded in these diseases.

The endogenous RISC complex is central to the silencing of genes by miRNAs and other small interfering RNAs such as small interfering RNAs (siRNAs). Interestingly, various therapeutic tools under development use miRNAs, siRNAs, or other antisense oligonucleotides (including miRNA-like backbones) that silence mHtt expression *in vivo* [1, 8]. The clinical testing of these compounds in the human brain will need to be carefully monitored for potential loss of RISC biological function. Much more work is required to understand better the role and impact of miRNA biogenesis abnormalities in HD and related trinucleotide disorders.

## Conclusions

In summary, we show that pre-miRNA to mature miRNA biogenesis is strongly compromised in the human HD striatum. This observation could help understand the pathological relationship between Htt-Ago2 binding *in vivo*. Furthermore, this study suggests that indirect or small changes in mature miRNA levels are insufficient to promote cell degeneration *per se* in trinucleotide diseases, compared to a “multiple-hit” scenario implicating deficits in miRNA biogenesis or other RISC-dependent mechanisms. This study, therefore, contributes to the ongoing debate about the contribution of mHtt in promoting selective cell death in the human brain [12, 14, 19, 25, 39, 45]. Our observations support the hypothesis that aggregated Htt is not directly correlated with neurodegeneration and that other cell, organ or system-dependent mechanisms are involved. Finally, the results herein will guide current and future therapeutic strategies involving the endogenous RISC in the human brain.

## Abbreviations

DGCR8: DiGeorge syndrome critical region 8; TRBP: Human immunodeficiency virus transactivating response RNA-binding protein; Ago2: Argonaute 2; RISC: RNA-induced silencing complex; NeuN: Neuronal nuclear protein; DARPP-32: Dopamine- and cAMP-regulated neuronal phosphoprotein; PSD95: Postsynaptic density protein 95; BDNF: Brain-derived neurotrophic factor; nt: Nucleotide.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40478-022-01407-7>.

**Additional file 1.** Supplementary Methods (Human blood samples).

**Additional file 2.** Supplementary Tables (List of antibodies).

**Additional file 3.** Supplementary Figures (Comparative analysis of protein expression between brain regions; Analysis of mRNA expression in HD brain; miRNA screening in HD brain; qRT-PCR analysis of normalization genes; Analysis of miRNA maturation in blood).

## Author contributions

The experiments with human samples were approved by the CHU de Québec local Research Ethics Committee (#2017-3017). All work with human subjects was approved by the CHU de Québec human ethics committee (#2020-4622)

and in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants. This work was supported by the Canadian Institute of Health Research (CIHR, grant #272311), the Fonds de Recherche en Santé (FRQS), and the Huntington's Disease Society of America (HDSA). The Harvard Brain Tissue Resource Center provided tissues and is supported in part by HHSN-271-2013-00030C. The authors would like to express a special appreciation to the nurses and staff who assisted in collecting and storing the human specimens. Finally, the authors are very grateful to all study participants and their families who have contributed to this study. All co-authors have provided consent for publication. S. P. and I. S.-A. participated in the study design and patient recruitment, experiments, data analysis, and writing, R. K., B. N., and A. L. participated in the experiments and data analysis, K. P. and J.J. participated in the study design and writing, M. L. participated in patient recruitment, S.S.H. planned and supervised the study, experiments, patient enrolment, and wrote the article. All authors participated in scientific discussions.

## Declarations

### Competing interests

The authors declare that they have no competing interests.

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Received: 24 May 2022 Accepted: 9 July 2022

Published online: 22 July 2022

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