Bioinformatic analysis of a microRNA regulatory network in Huntington's disease

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Huntington's disease is an autosomal dominant hereditary neurodegenerative disease characterized by progressive dystonia, chorea and cognitive or psychiatric disturbances. The leading cause is the Huntington gene mutation on the patient's chromosome 4 that produces a mutated protein. Recently, attention has focused on the relationship between microRNAs and Huntington's disease's pathogenesis. In Huntington's disease, microRNAs can interact with various transcription factors; dysregulated microRNAs may be associated with the Cytosine deoxynucleotide-Adenine ribonucleotides-Guanine ribonucleotide trinucleotide repeat sequence, which encodes multiple glutamine residues in the gene encoding the huntingtin (Htt) protein (Hu et al., 2010). Over the years, a large amount of literature has described the probable molecular mechanisms associated with neuronal dysfunction and degeneration in HD, including misfolding aggregation and the decreased clearance of mutant Htt (mHtt), autophagy, transcriptional dysregulation, mitochondrial dysfunction, and a damaged ubiquitin-proteasome system (McColgan and Tabrizi, 2018). Among various molecular changes in HD, understanding gene expression dysregulation may reveal possible pathogenesis and provide potential therapeutic HD strategies.

MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs found in eukaryotes that have regulatory functions. They are about 20-25 nucleotides long and identify each target mRNA through complementary base pairing. In accordance with the degree of complementarity, differently guided silencing complexes target mRNA degradation or translation repression of the target mRNA (Rodriguez, 2004; Saraiva et al., 2017). MicroRNAs are expressed in all tissues and are thought to regulate around 30% of the entire human genome (Gednic et al., 2013). In the central nervous system, miRNAs appear to have the greatest diversity (Thomson et al., 2004). Emerging studies have shown that miRNAs may play a critical role in the pathogenesis and development of neurodegenerative disorders (Rinchetti et al., 2018). Indeed, several miRNAs can interact with several transcriptional co-factors involved in HD, including RelA/NF-κB, p53 (Ghose et al., 2011), Mitofusin2 (Bucha et al., 2015), Tata Binding Protein (TBP) (Sinha et al., 2010), REST, and REI (Johnson et al., 2008). The level of miRNA dysregulation may correlate with the length of the CAG repeat in the mHtt allele and be involved in the progression or severity of HD (Langfelder et al., 2018). MiR-146a could target TBP, so dysregulation of TBP by miRNA-146a may contribute to HD pathogenesis (Sinha et al., 2010). Reynolds et al. (2018) found...
the decreased levels of miRNA-34a-5p could increase p53 protein levels in brain tissue from R6/2 mice and promote progression in an HD model. These results suggested that dysregulated neuronal miRNAs might be related to the pathogenesis of HD. Moreover, modulating the expression of miRNAs could also exert therapeutic effects. It was suggested that miR-124 might slow down HD’s progression through its essential role in neuronal differentiation and survival (Kim et al., 2015). Ban et al. (2017) suggested that miR-27a could reduce the HD cell’s mHtt level by augmenting multidrug resistance protein-1 (MDR-1) function.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs greater than 200 nt in length and similar in gene structure to miRNAs (Wang and Chang, 2011). LncRNAs have also been reported to participate in many important biological processes, including transcriptional and post-transcriptional regulation (Mercer et al., 2009). Several studies have shown dysfunctional or mutated lncRNAs are related to the pathogenesis of HD (Cheng et al., 2013). Chanda et al. (2018) reported that the Neat1, X-inactive specific transcript (Xist) and Meg3 showed a significant increase in HD cell and animal models. Knock-down of Meg3 and Neat1 could down-regulate endogenous Tp53 levels and reduce aggregates formed by mHtt in all models of HD. Sunwoo et al. (2017) demonstrated the NEAT1 was up-regulated in HD animal model, which may contribute to the neuroprotective mechanism against neuronal injury. Overexpression of Abhd11os could produce neuroprotection against an N-terminal fragment of mHtt, which indicated that the loss lncRNA Abhd11os probably contributes to striatal vulnerability in HD (Francelle et al., 2015). Therefore, investigating lncRNA’s HD role is essential for understanding HD’s pathogenesis and providing possible therapeutic strategies.

This study used data from Hoss et al. (2015) for bioinformatics analysis, who analyzed different miRNA expression profiles from the brains of 26 HD patients, two asymptomatic HD patients and 36 normal controls. According to the identified differential expression of miRNAs, we predicted target genes and related lncRNAs and constructed a miRNA-centered integrated regulatory network. Pathway and functional enrichment analyses of target genes were analyzed using a database for annotation and visualization and comprehensive discovery software. Our purpose was to further explore the involvement of miRNAs in the pathogenesis of HD and to attempt to elucidate the comprehensive miRNA regulatory network in HD.

### Table 1. Differentially expressed miRNAs list

| Name               | State | Sequence                        | log2FC | P-value  | Chromosome location |
|-------------------|-------|---------------------------------|--------|----------|---------------------|
| hsa-miR-10b-5p    | Up    | uacccuguaacngaauauugug          | 3.94   | 1.28 x 10^-20 | chr2: 176530403-176530412 [+]
| hsa-miR-196a-5p   | Up    | uagguaugaucauguauugugg           | 2.35   | 2.97 x 10^-20 | chr1: 48632460-48632559 [-]
| hsa-miR-615-3p    | Up    | ucgaccuccggucucccecuu            | 1.59   | 3.23 x 10^-16 | chr2: 54033940-54034045 [+]
| hsa-miR-10b-5p    | Up    | acaagnaucaauguguggggau           | 1.45   | 2.13 x 10^-12 | chr2: 17653034-176530412 [+]
| hsa-miR-196b-5p   | Up    | uagguauguuuccuguuguuggg          | 1.31   | 2.33 x 10^-8  | chr7: 2769480-2769563 [-]
| hsa-miR-483-5p    | Up    | agacgggaggaagaagaggg             | 1.16   | 1.6 x 10^-3  | chr11: 234347-2343490 [-]
| hsa-miR-144-3p    | Up    | uacgauaugaaugauaguauu            | 1.08   | 9.16 x 10^-6  | chr17: 28861533-28861668 [-]
| hsa-miR-4449      | Down  | gucgcggggggcggccgggccccca       | 1.09   | 5.0 x 10^-4  | chr4: 52712682-52712747 [-]
| hsa-miR-4488      | Down  | gacggccgggcaacccggg             | 1.32   | 2.0 x 10^-3  | chr11: 61528596-61528637 [+]

Abbreviations: miRNA, microRNA; FC, Fold Change.

![Fig. 1. Volcano diagrams of 9 significantly differentially expressed micro (mi) RNA after FDR-adjustment. Points labeled red were up-regulated and points labeled as blue were down-regulated in HD.](http://targetscan.org) (Lewis et al., 2003), PicTar (http://pictar.bio.nyu.edu) (Krek et al., 2005), miRanda (http://microrna.sange.r.ac.uk) (Enright et al., 2004), miRWalk (http://mirwalk.uni-h)

### 2. Materials and methods

#### 2.1 Data source and pre-processing

The data in this study were extracted from Hoss et al. (2015). This investigation aimed to explore the possible mechanisms of dynamic regulation of miRNAs affecting HD gene expression and altering HD progression and severity. They conducted next-generation miRNA sequence analysis on the prefrontal cortex (Brodmann Area 9) of 26 HD patients, two asymptomatic HD patients, and 36 controls. Finally, 75 miRNAs that were differentially expressed in the HD brain (false discovery rate P-value < 0.05) were identified. We further screened nine miRNAs according to our criteria, where an adjusted P < 0.05 and |log_2 fold-change (FC)| > 1 were used as thresholds. Based on the screening of miRNAs, a regulatory network was constructed to further elucidate the miRNAs’ roles in HD’s pathogenesis.

**Target gene prediction.** Five public algorithms predicted the target genes regulated by miRNAs, including TargetScan (http://targetscan.org) (Lewis et al., 2003), PicTar (http://pictar.bio.nyu.edu) (Krek et al., 2005), miRanda (http://microrna.sange.r.ac.uk) (Enright et al., 2004), miRWalk (http://mirwalk.uni-h)
2.2 miRNA-IncRNA regulatory relationship construction

The StarBase v2.0 (http://starbase.sus.edu.cn/) was developed to decipher protein-RNA and miRNA-target interactions, such as protein-IncRNA, miRNA-IncRNA, miRNA-mRNA interactions, and networks from a 108 Cross-linking Immunoprecipitation combined with a high-throughput Sequencing dataset. StarBase also provided miR function web tools to analyze non-coding (nc) RNAs (miRNAs, IncRNAs) and target genes in miRNA-centered regulatory networks. In this study, the interaction networks of miRNA-IncRNAs related to the nine differentially expressed miRNAs were extracted from starBase v2.0 and reserved for further analysis.

2.3 Pathway and functional enrichment analysis of target genes

The Database for Annotation Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov) (Huang et al., 2009) online analysis tool constitutes a comprehensive bioinformatics database. This system can mine the biological functions of many genes and play a key role in further extracting biological, genetic information. The Gene Ontology Database (GO; www.Geneontology.org) describes the basic characteristics of genes and their products. Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg) uses DAVID to perform pathway enrichment analysis on miRNAs targeting genes. P < 0.05 was considered statistically significant.

2.4 Construction of miRNA regulatory network and visualization analysis

Cytoscape is an open-source biological information analysis software that can build a visual network diagram of molecular interactions. The connection between nodes indicates interaction between each other and can integrate existing gene expression information into the network diagram. So it is easier to observe the correlation between protein and protein (Smoot et al., 2011). We reserved and obtained miRNA-IncRNA and miRNA-mRNA interaction networks and subsequently visualized these using Cytoscape. The GO plot package in R language was used to visualize the GO and KEGG results of target genes (Walter et al., 2016).

3. Results

3.1 Identification of differentially expressed miRNAs

An adjusted P < 0.05 and |log₂ FC| > 1 were set as thresholds. A total of nine differentially expressed miRNAs were identified, including seven up-regulated and two down-regulated HD patients compared with healthy controls Table 1 and Fig. 1.

3.2 miRNA-mRNA and miRNA-IncRNA comprehensive regulatory network

According to predicted miRNA-mRNA and miRNA-IncRNA regulatory networks, a miRNA-centered, comprehensively regulated network was constructed (Fig. 2). The network showed the hsa-miR-144-3p regulated the largest number of IncRNAs, including XIST and taurine upregulated gene 1 (TUG1). The miRNAs, hsa-miR-196a-5p and hsa-miR-10b-5p, regulated most target genes, including class I homeobox (HOX) and brain-derived neurotrophic factor (BDNF).

3.3 Function enrichment analysis of hsa-miR-196a-5p target genes

Sixty-nine target genes of hsa-miR-196a-5p were identified by performing GO enrichment analysis. We found such genes were mostly enriched in 59 GO terms, such as anterior/posterior pattern specification (P = 4.80 × 10⁻⁷), sequence-specific DNA binding (P = 2.97 × 10⁻⁵), and transcription from the RNA polymerase II promoter (P = 1.04 × 10⁻¹). The top 10 functions enriched for target genes are listed in Table 2; 18 GO terms (P < 0.01) are shown in Fig. 3. Pathway enrichment analysis for the target genes of hsa-miR-196a-5p was mainly enriched in the gonadotropin-releasing hormone (GnRH) signaling pathway (P = 0.002), neurotrophin signaling pathway (P = 0.004) and insulin signaling pathway (P = 0.006) (Table 3, Fig. 4).

3.4 Hsa-miR-10b-5p target genes GO enrichment analysis

Fifty-eight target genes of hsa-miR-10b-5p were identified, which were mainly enriched in nucleus (P = 6.37 × 10⁻⁴), nucleoplasm (P = 1.55 × 10⁻⁵), and transcription, DNA-templated (7.78 × 10⁻⁴) (Table 4, Fig. 5).

3.5 Targeted IncRNAs of multiple differentially expressed miRNAs

By retrieving from starBase v2.0, five of nine differentially expressed miRNAs possessed a regulatory relationship with IncRNAs. We found hsa-miR-144-3p regulated the largest number of IncRNAs, including XIST and TUG1. By analyzing the regulatory network, we found some of the IncRNAs were targeted by multiple differentially expressed miRNAs, such as XIST, TUG1 and GS1-358P8.4. LncRNAs targeted by at least two miRNAs are shown in Table 5.

4. Discussion

HD is an autosomal dominant neurodegenerative disease caused by CAG repeated amplification of the Htr1c; however, HD's exact pathophysiology remains obscure. Although a large number of early investigations have focused on the effect of transcriptional dysregulation of gene expression on HD, recent studies have shown that post-transcriptional mechanisms are involved in the pathogenesis of HD (Juźwik et al., 2019). Specifically, neuronal miRNAs have been shown to participate in the post-transcriptional regulation of target genes (Minarikova et al., 2018). The results from Hoss et al. (2015) are one of the latest and most important contributions to this field. They performed an unbiased analysis of next-generation miRNA sequence in the prefrontal cortex (Brodmann Area 9) of brains from 26 HD patients, two asymptomatic HD patients, and 36 controls. Seventy-five differentially expressed miRNAs were identified in the HD brain. Their results enabled us to focus on several miRNAs using more stringent screening criteria. In the present study, we chose an adjusted P < 0.05 and |log₂ FC| > 1 as thresholds and identified nine differentially expressed miRNAs associated with HD. We consequently constructed a miRNA-centered comprehensive regulatory network. To our best knowledge, this was the first time that a regulatory network study was constructed containing IncRNAs in HD. Of these, hsa-miR-196a-5p and hsa-miR-10b-5p were highly and significantly regulated; they also had the largest number of target genes.
Fig. 2. A micro(mi)RNA-centered comprehensive regulatory network. The rhombus represents miRNAs (red represents up-regulated, green represents down-regulated); pink circles represent target genes; blue triangles represent long non-coding (lnc)RNAs.

Fig. 3. To understand the biological processes, cell components, and molecular functions involved in target genes of (hsa)-miR-196a-5p. The GO gene enrichment analysis of target genes was obtained, as shown in (Fig. 3) (terms containing more genes tended to have a more significant $P$-value).

It has been reported the hsa-miR-196a participates in the pathogenesis and progression of HD (Cheng et al., 2013). Fu et al. (2015) believed hsa-miR-196a could alter the RIG-I-like receptor signaling pathway and change certain well-defined pathways expression in HD, such as cell adhesion and apoptosis. In this study, we found hsa-miR-196a-5p mainly interacted with HOX genes, which had been reportedly involved indirectly in the neuroprotective response in HD. Hoss et al. (2014) reported that increased HOX genes expression could enhance H3K27me3 or reduce Polycomb G group (PcG) repression; their findings suggested the possibility of increased miRNAs and HOX genes expression might be associated with enhanced H3K27me3 or reduced PcG repression, and that hsa-miR-196a-5p might be participated in the progression of HD by regulating HOX genes expression. Through
Fig. 4. Pathway enrichment analysis for the target genes of hsa-miR-196a-5p was shown in (Fig. 4), which is mainly enriched in the gonadotropin-releasing hormone GnRH signaling, Neurotrophin signaling and Insulin signaling pathways (terms containing more genes tended to have a more significant P-value).

function enrichment analysis of hsa-miR-196a-5p target genes, we found that most genes were enriched in protein binding, the nucleus, the regulation of transcription from the RNA polymerase II promoter, and transcription factor activity. Such conclusions are consistent with previous research findings that suggested that the transcriptional dysregulation of gene expression might be one of the main pathogenic changes, which confirmed the role of hsa-miR-196a-5p in HD. Cheng et al. (2013) suggested that miR-196a suppresses mHtt directly in the brain and improves neuropathological progression. MiR-196a could also suppress the RAN binding protein 10 (RANBP10) expression by binding to its 3’ UTR, which could exacerbate neuronal morphology and intracellular transport. Also, miR-196a might enhance neuronal morphology by suppressing RANBP10 and increasing the ability of β-tubulin polymerization, suggesting its therapeutic effects in HD patients (Her et al., 2017). Furthermore, overexpression of miR-196a could surpass the expression of apoptosis-related genes and improve mitochon-
Table 2. Gene ontology enrichment analysis for the target genes of hsa-miR-196a-5p (top 10)

| ID       | Description                                      | P-value         | Total no. of genes | Genes                                      |
|----------|--------------------------------------------------|-----------------|--------------------|--------------------------------------------|
| GO:000952| anterior/posterior pattern specification          | $4.80 \times 10^{-7}$ | 7                  | HOXC8, HOXB7, HOXA5, OTX1, HOXA7, HOXB6, HOXA9 |
| GO:0043565| sequence-specific DNA binding                     | $2.97 \times 10^{-6}$ | 12                 | BACH1, ZNF516, HOXC8, HOXB7, HOXA5, OTX1, HOXA7, HOXB6, HOXA9, PBX3, NR2C2, FOXP2 |
| GO:006366| transcription from RNA polymerase II promoter     | $1.04 \times 10^{-4}$ | 10                 | BACH1, ZNF516, HAND1, HOXA5, GATA6, OTX1, HOXA7, HMGA2, PBX3, HMGA1 |
| GO:0048704| embryonic skeletal system morphogenesis           | $3.98 \times 10^{-4}$ | 4                  | HOXB7, HOXA5, HOXA7, HOXB6 |
| GO:0005515| protein binding                                  | $5.34 \times 10^{-4}$ | 47                 | BACH1, ZMYND11, COL3A1, NAP1L1, CASK, EEA1, IGF2BP3, LIN28B, NR2C2, HOOK1, PEG10, HAND1, GATA6, DDX19A, HOXA5, DDX19B, MAP5K1, HOXA9, RANBP2, C11orf57, USP15, OTX1, CCDC47, BIRC6, SOCS4, GAN, RICTOR, HMGA2, HMGA1, FNIP1, FOXP2, MAP4K3, EPS15, EPHA7, CDKN1B, RCC2, CEPP50, HOXB7, RIOK3, SMARCC1, HOXB6, HABP4, CALM3, COL1A1, LCOR, PPP1R15B, CALM1 |
| GO:0031901| early endosome membrane                           | $7.38 \times 10^{-4}$ | 5                  | EPS15, SLC9A6, RCC2, SNX16, EEA1 |
| GO:0003700| transcription factor activity, sequence-specific DNA binding | $7.75 \times 10^{-4}$ | 12                 | BACH1, ZNF516, HOXC8, HOXB7, HOXA5, GATA6, OTX1, HOXB6, PBX3, HMGA1, NR2C2, FOXP2 |
| GO:0045944| positive regulation of transcription from RNA polymerase II promoter | $8.35 \times 10^{-4}$ | 12                 | BACH1, HAND1, HOXA5, GATA6, OTX1, SMARCC1, HOXA7, CASK, HMGA2, PBX3, HMGA1, NR2C2 |
| GO:0045022| early endosome to late endosome transport nucleus | $2.0 \times 10^{-3}$ | 3                  | HOOK1, SNX16, EEA1 |
| GO:0005634| nucleus                                           | $3.0 \times 10^{-3}$ | 31                 | BACH1, ZMYND11, ZNF516, NAP1L1, IGF2BP3, NR2C2, PEG10, HAND1, GATA6, DDX19A, HOXA5, DDX19B, HOXA7, HOXB9, USP15, CCN1, OTX1, HMGA2, HMGA1, FOXP2, CDKN1B, RCC2, HOXB7, HOXB6, CALM3, HABP4, CPD, PBX3, LCOR, CALM1, RANBP10 |

Abbreviations: GO, Gene Ontology.
drial morphology and activity by upregulating CREB Binding Protein (CBP) and peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1α) expression (Kunkanjanawan et al., 2016).

Levels of plasma hsa-miR-10b-5p are significantly up-regulated in HD patients compared with asymptomatic HD gene carriers and healthy controls (Hoss et al., 2017). Hoss et al. (2015) confirmed that hsa-miR-10b-5p showed the most prominent associations with age of onset, disease stage, and the extent of neuropathological impairment in HD patients. In many target genes, we found brain-derived neurotrophic factor (BDNF) was one of the target genes of hsa-miR-10b-5p, critical to striatal neurons (Zuccato et al., 2003). Reduced regulation of BDNF transcription in the cerebral cortex by the dysregulated expression of hsa-miR-10b-5p might be a leading candidate mechanism for striatal neuronal death in HD (Buckley and Johnson, 2011). Other genes, such as BAZ2B, are also thought to be related to the pathogenesis of HD (Maulik et al., 2018). Analysis of pathway enrichment for the target genes of hsa-miR-10b-5p showed that this miRNA targeted significantly enriched pathways in HD involved in the three pathways, including GnRH, neurotrophin, and insulin signaling pathways. Other involved miRNAs in the present study, including hsa-miR-10b-3p, hsa-miR-144-3p, hsa-miR-483-5p, hsa-miR-4488 had also been reported to be differentially expressed in HD samples; hsa-miR-10b-3p was considered to have a significant relationship with the CAG length of HD (Hoss et al., 2015).

LncRNA is a type of RNA with a length greater than 200 nucleotide units. Because there is no open reading frame (ORF), it is called non-coding RNA (Iyer et al., 2015). These show epigenetic, transcriptional, and post-transcriptional gene expression (Wapinski and Chang, 2011). Since the 1990s, several individual lncRNAs have been studied; however, the exact mechanisms underlying transcriptional regulation by lncRNAs are not precise. Recently, numerous studies have suggested that thousands of lncRNAs might interact with multiple inhibitory chromatin regulatory complexes, including PRC2, SM CX and RCOR1, and have shown that lncRNAs are involved in numerous neurodegenerative disorders, including HD (Khalil et al., 2009). Other lncRNAs have been shown to affect the function of transcription factors (TFs), either by directly inactivating TFs or promoting their export from the nucleus (Willingham, 2005).

Some of the lncRNAs have been confirmed involved in the pathogenesis of HD. Chung et al. (2011) found that the repeat expansion reduced HTT-AS_v1 expression; the levels of HTT-AS_v1 were reduced in the human HD frontal cortex. Also, HTT-AS_v1 could negatively regulate Htt expression in a repeat length-dependent manner. It was known that the levels of BDNF were down-regulated in the brains of HD patients, possibly contributing to the clinical characteristics of HD. BDNF-AS, a lncRNA transcribed from the BDNF opposite strand, could inhibit BDNF tran-5

| ID          | Description                  | P-value | Total no. of genes Genes                  |
|-------------|------------------------------|---------|------------------------------------------|
| hsa04912    | GnRH signaling pathway       | 0.002   | 4                                         |
| hsa04722    | Neurotrophin signaling pathway | 0.005   | 4                                         |
| hsa04910    | Insulin signaling pathway    | 0.007   | 4                                         |
| hsa04921    | Oxytocin signaling pathway   | 0.01    | 4                                         |
| hsa05214    | Glion                         | 0.01    | 3                                         |
| hsa04720    | Long-term potentiation       | 0.02    | 3                                         |
| hsa04915    | Estrogen signaling pathway   | 0.03    | 3                                         |
| hsa04916    | Melanogenesis                | 0.03    | 3                                         |

Abbreviations: GO, Gene Ontology.

Table 3. Pathway enrichment analysis for the target genes of hsa-miR-196a-5p

According to our network, several lncRNAs were regulated by differentially expressed miRNAs, including XIST and TUG1. XIST is an lncRNA that is a necessary condition for female X chromosome silencing in placental mammals. XIST spreads from one of its X chromosome transcription sites to gradually cover it; it also interacts with the PRC2 complex, deposits suppressive histone markers, and effectively suppresses gene transcription. Previous studies concluded that the dysregulation of XIST in HD might directly cause changes in one or a few proximal target genes (Johnson, 2012). Chanda et al. (2018) also identified the XIST was a significant increase in HD cell and animal models. TUG1 is highly expressed in mammalian brains. Initially found during genomic screening, the TUG1 gene was up-regulated during taurine-treated retinal cell development. The expression of TUG1 was down-regulated in HD patients (Johnson, 2012). Khalil et al. (2009) found in their research that TUG1 was a direct downstream target and a regulator of p53; it was up-regulated in HD to counterbalance mHtt cytotoxicity pro-survival factor in neurons to activate p53 (Zhang et al., 2014).
| ID         | Description                                                                 | P-value          | Total no. of genes | Genes                                                                 |
|------------|------------------------------------------------------------------------------|------------------|--------------------|----------------------------------------------------------------------|
| GO:0005654 | nucleoplasm                                                                  | $1.55 \times 10^{-5}$ | 23                 | ZMYND11, JARID2, E2F7, BBX, ZNF367, ELAVL2, RORA, DAZAP1, CTNNBP1, NONO, HOXA3, WDR26, BAZ1B, CEP350, GATA6, MTF2, NCOA6, BCL6, H3F3B, TFAP2C, RPRD1A, NCOR2, GOLGA3 |
| GO:0003682 | chromatin binding                                                             | $3.62 \times 10^{-5}$ | 9                  | NONO, ZMYND11, BAZ1B, JARID2, GATA6, NCOA6, BCL6, NCOR2, HOXD10       |
| GO:000977  | RNA polymerase II regulatory region sequence-specific DNA binding            | $5.71 \times 10^{-5}$ | 7                  | ZMYND11, JARID2, GATA6, E2F7, BCL6, TFAP2C, RORA                     |
| GO:0008584 | male gonad development                                                        | $2.19 \times 10^{-4}$ | 5                  | GATA6, H3F3B, TFAP2C, SIX4, BCL2L11                                   |
| GO:0048538 | thymus development                                                            | $2.35 \times 10^{-4}$ | 4                  | HOXA3, JARID2, SIX4, BCL2L11                                        |
| GO:0000122 | negative regulation of transcription from RNA polymerase II promoter nucleus  | $3.79 \times 10^{-4}$ | 10                 | HOXB3, ZMYND11, JARID2, GATA6, MTF2, E2F7, KLF11, BCL6, TFAP2C, NCOR2 |
| GO:0005634 | nucleus                                                                       | $6.37 \times 10^{-4}$ | 30                 | ZMYND11, E2F7, ZNF367, RORA, CNOT6, HOXD10, DAZAP1, NONO, HOXA1, ARH2, HOXA3, FIGN, GATA6, RNF165, TIAM1, BCL6, BAZ2B, GOLGA3, JARID2, KLF11, SIX4, CTNNBP1, HOXB3, MTF2, NCOA6, CELF2, H3F3B, TFAP2C, XRN1, NCOR2 |
| GO:0006351 | transcription, DNA-templated                                                  | $7.78 \times 10^{-4}$ | 16                 | ZMYND11, JARID2, E2F7, BBX, ZNF367, RORA, CNOT6, HOXD10, NONO, HOXB3, HOXA1, HOXA3, BAZ1B, BCL6, BAZ2B, NCOR2 |
| GO:0009952 | anterior/posterior pattern specification                                       | $2.0 \times 10^{-3}$  | 4                  | HOXB3, CTNNBP1, HOXA3, HOXD10                                       |
| GO:0003700 | transcription factor activity, sequence-specific DNA binding                 | $3.0 \times 10^{-3}$  | 10                 | HOXB3, HOXA3, GATA6, E2F7, KLF11, ZNF367, BCL6, TFAP2C, RORA, HOXD10 |

Abbreviations: GO, Gene Ontology.
Table 5. LncRNAs targeted by multiple (≥ 2) differential miRNAs

| LncRNAs | The number of miRNAs regulates the lncRNA |
|---------|------------------------------------------|
| XIST    | 5                                        |
| TUG1    | 3                                        |
| G5S1-35S8P.4 | 3                                    |
| RP11-20G6.3 | 3                                    |
| LINCO0657 | 2                                        |
| DCP1A  | 2                                        |
| RP6-24A23.7 | 2                                    |

Abbreviations: miRNA, microRNA, lncRNAs, long non-coding RNAs.

5. Conclusions
Although extensive studies have revealed the abnormal expression of miRNAs in HD, its pathological significance's exact molecular mechanisms remain unclear. Using the expression profile data of Hoss et al. (2014), a comprehensive regulatory network centered on miRNAs was constructed. We identified that hsa-miR-196a-5p and hsa-miR-10b-5p were significantly differentially expressed, which regulated most target genes. Targeted genes, including HOX and BDNF, and targeted lncRNAs, including XIST and TUG1, may play important roles in HD's development. Research in this area may help deepen our understanding of the role of miRNAs in HD's pathogenesis.

Author contributions
This manuscript was primarily written by ZW. Figures were produced by XD and ZW. SC contributed to editing the review and contributed to the review revision. All authors read and approved the final manuscript.

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Conflicts of Interest
All authors have no conflicts of interest to declare.

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