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Review

# Long non-coding RNAs in Huntington's disease neurodegeneration

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ABSTRACT

Neurodegeneration in the brains of Huntington's disease patients is accompanied by widespread changes in gene regulatory networks. Recent studies have found that these changes are not restricted to protein-coding genes, but also include non-coding RNAs (ncRNAs). One particularly abundant but poorly understood class of ncRNAs is the long non-coding RNAs (lncRNAs), of which at least ten thousand have been identified in the human genome. Although we presently know little about their function, lncRNAs are widely expressed in the mammalian nervous system, and many are likely to play critical roles in neuronal development and activity. LncRNAs are now being implicated in neurodegenerative processes, including Alzheimer's (AD) and Huntington's disease (HD). In the present study, I discuss the potential significance of lncRNAs in HD. To support this, I have mined existing microarray data to discover seven new lncRNAs that are dysregulated in HD brains. Interestingly, several of these contain genomic binding sites for the transcriptional repressor REST, a key mediator of transcriptional changes in HD, including the known REST target lncRNA, DGCR5. Previously described lncRNAs TUG1 (necessary for retinal development) and NEAT1 (a structural component of nuclear paraspeckles) are upregulated in HD caudate, while the brain-specific tumour-suppressor MEG3 is downregulated. Three other lncRNAs of unknown function are also significantly changed in HD brains. Many lncRNAs regulate gene expression through formation of epigenetic ribonucleoprotein complexes, including TUG1 and MEG3. These findings lead me to propose that lncRNA expression changes in HD are widespread, that many of these result in altered epigenetic gene regulation in diseased neurons, and that contributes to neurodegeneration. Therefore, elucidating lncRNA network changes in HD may be important in understanding and treating this and other neurodegenerative processes.



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## Pathogenic gene regulation caused by mutant Huntingtin

Huntington's disease (HD) is a dominantly-inherited neurodegenerative condition with symptoms of chorea, psychiatric problems, and dementia. Neurodegeneration is most pronounced in the striatum, where medium spiny neurons are lost, but other regions such as the cortex are also affected to a lesser degree (Zuccato et al., 2010). The disease is inevitably fatal, and no drugs presently exist to treat it. The frequency of HD is approximately 1 in 10,000 and symptoms generally appear in middle age. HD belongs to the intriguing group of trinucleotide repeat disorders, where an expansion in a trinucleotide

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repeat region within a single gene is pathogenic. In the case of HD, the repeat occurs within the first exon of the gene called Huntingtin (HTT), which encodes a ubiquitously-expressed 3144 amino acid protein of unknown function, leading to a toxic gain of function in the mutant protein (mutHTT) (MacDonald et al., 1993). Healthy individuals possess HTT alleles containing a CAG-repeat region of <36 in length, encoding a polyglutamine tract in the translated Htt protein. Carriers of HTT alleles with longer CAG repeats manifest various severities of HD phenotype, dependent on the length of the repeat (Stine et al., 1993). The mechanisms through which this simple mutation in a ubiquitous protein results in such a cell-specific neurodegeneration have been the focus of intense research for two decades.

Since the discovery of the HTT gene, many advances have been made in understanding how the mutant protein results in neurodegeneration, through both cell autonomous (i.e. within the cell) and non-autonomous (i.e. between cells) processes. The latter includes well-documented toxic changes that take place in the interactions between neurons, including increased sensitivity to excitotoxicity through the loss of wild-type Htt (Leavitt et al., 2006), and disruption of trophic support between neurons (Zuccato et al., 2001). Cell autonomous candidates comprise the formation of intracellular protein aggregates (although this in fact may be a protective mechanism) (DiFiglia et al., 1997), mitochondrial dysfunction (Panov et al., 2002), and various changes to gene regulatory networks (Johnson and Buckley, 2009). Through alterations in physical properties of the HTT protein, the polyQ region promotes the interaction of HTT with other proteins including various transcription factors (Cha, 2007), resulting in their incorporation in intracellular inclusion bodies. The same mutation also diminishes the ability of HTT to carry out its usual roles in intracellular cargo transport, fundamental for neuronal function. It is also likely that wild-type HTT is neuroprotective, and loss of one allele sensitizes neurons to toxicity of the mutant allele (reviewed in Zuccato and Cattaneo, 2005). In addition to these abnormalities in cellular function, mutHTT leads to profound changes in transcriptional and post-transcriptional processes in cells where it is expressed. Focussed studies, then large-scale microarray analyses, have consistently shown that human HD patients and mouse models experience major alterations in brain mRNA levels (Augood et al., 1996; Hodges et al., 2006). The severity of transcriptomic changes reflects the severity of neurodegeneration, with the striatum experiencing the starkest changes followed by more moderate changes in the cortex, and relatively mild changes in the cerebellum (Hodges et al., 2006). Similar studies have also been carried out in a range of mouse models, with results that are reasonably consistent with human (Kuhn et al., 2007).

Quite a number of transcription factors have been identified that may mediate the observed gene expression phenotype caused by mutHTT, including the tumour suppressor p53 (Bae et al., 2005), the zinc finger factor SP1 (Zhai et al., 2005), TATA-binding protein (TBP) (van Roon-Mom et al., 2002), the sterol responsive element binding protein (SREBP) (Valenza et al., 2005), CBP, a cofactor of the neuronal excitatory factor CREB (Nucifora et al., 2001), and the master neuronal regulator REST (RE1 Silencing Transcription Factor) (Zuccato et al., 2003). The pathways may either experience altered affinity of a naturally-occurring interaction of HTT protein with the transcription factor, or may result from a gain-of-function interaction conferred by polyQ expansion. In the case of TBP, the polyQ expansion increases the natural affinity of HTT, resulting in sequestration of this protein into intracellular inclusions (van Roon-Mom et al., 2002). Similarly for p53, mutHTT has increased affinity, raising nuclear levels of p53 and also stimulating its transcription (Bae et al., 2005). In the case of REST, mutHTT displays decreased affinity, and elevated nuclear localisation of REST occurs (Zuccato et al., 2003). In addition, elevated expression of REST mRNA in HD may aggravate this process (Ravache et al., 2010). mutHTT also enters the nucleus and directly regulates gene transcription in a polyQ-dependent manner, either by binding to chromatin directly (Benn et al., 2008), or by interacting with various core components of the RNA Pol II complex (van Roon-Mom et al., 2002; Zhai et al., 2005). Given this wealth of suspects, the challenge is to define which of the many potential downstream target genes and pathways actually contribute to neuronal dysfunction and death.

Amongst the REST targets is the gene encoding the neurotrophic factor BDNF (Timmusk et al., 1999). The striatum, site of the most severe neurodegeneration in HD, depends on BDNF transported from the cortex by corticostriatal afferents (Altar et al., 2008). The reduction in this transport, resulting from increased BDNF gene repression by REST in the cortex, is a promising explanation for the specific loss of striatal neurons in HD, where the mutHTT protein is ubiquitously expressed (Baquet et al., 2004; Zuccato et al., 2003). Nevertheless, the pathological action of REST in HD is probably not restricted to the BDNF gene alone — many hundreds of REST target genes are repressed as a result of mutHTT (Zuccato et al., 2007). It is possible that the repression of some or many of these genes contributes to HD neurodegeneration in addition to BDNF. The study of transcriptional dysregulation of REST in HD has been particularly tractable, since we have powerful experimental and bioinformatic tools for understanding the genomic targeting of this factor, although our methods to predict target genes from this information remain crude (Johnson et al., 2006; Yu et al., 2011). Thus it is possible to identify the total set of REST binding sites in the human and mouse genomes, incorporating both information on its preferred DNA sequence binding element (the RE1, or Repressor Element 1) and experimental mapping based on the high throughput Chromatin Immunoprecipitation with Next Generation Sequencing (ChIPSeq) (Johnson et al., 2007; Yu et al., 2011). A simple comparison of the known REST target genes – defined as those containing a ChIPSeq-identified REST binding site – to those genes changing in HD as measured by microarray, showed that these target genes are strongly clustered amongst the most highly repressed in HD (Johnson and Buckley, 2009) - implying that changes in REST function are responsible for many of the gene expression changes in HD brains. The gene expression changes occurring in HD are non-random, in terms of the functions of the genes that change: in general, genes specific to the nervous system are repressed in HD. This suggests that HD neurons undergo major transcriptional changes in their neuronal specific gene regulatory programme. Thus, by applying these genomic methods to REST in HD, it is possible to gain insights into pathogenic changes that take place in neuron-specific gene networks, and in principle, to identify new gene expression pathways in the disease state.

## Non-coding RNA pathways in Huntington's disease

In the past decade, we have come to appreciate that the human genome contains many thousands of non-coding RNA (ncRNA) genes (Lipovich et al., 2010). Numerous classes of ncRNAs have been discovered, ranging from various small RNA species at 17–22nt, to the 19 kb X-chromosome inactivating RNA *XIST* (Lipovich et al., 2010). While they play diverse roles within the cell, it does appear that the majority of ncRNAs are devoted in some way to gene regulation, either transcriptional, post-transcriptional or stable epigenetic gene regulation. The mammalian nervous system is particularly rich in ncRNAs. A large fraction of ncRNAs are expressed specifically in the nervous system, or during its development, or in specific neuronal subpopulations (Mercer et al., 2008, 2010).

The best understood ncRNA system is post-transcriptional mRNA regulation by the microRNAs (or “miRNAs”) (Bartel, 2009). MicroRNAs have heralded a revolution in our understanding of the complexity of eukaryotic gene regulation since their discovery in the 1990s. The elegance and unity of their mechanism of action have facilitated our understanding of this key ncRNA pathway, although unfortunately these qualities probably do not extend to other ncRNA