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Review

Transcriptional Regulation of the Huntingtin Gene

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Abstract. Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide expansion in the *HTT* gene, which encodes for an abnormal polyglutamine tract in the huntingtin protein (HTT). This review examines the known mechanisms of *HTT* gene regulation. We discuss *HTT* expression patterns, features of the *HTT* promoter, regulatory regions of the *HTT* promoter with functional significance, and *HTT* regulators located outside of the proximal promoter region. The factors that influence *HTT* expression in the brain and the mechanisms of *HTT* transcriptional regulation are currently poorly understood, despite continuing research. Expanding knowledge of *HTT* regulation will inform future studies investigating HTT function. Improving understanding of *HTT* expression and control may also uncover novel therapeutic approaches for HD through the development of methods to modulate mHTT levels.

Keywords: Huntington's disease, transcription, regulation of gene expression, transcriptional regulatory elements, promoter regions

INTRODUCTION

Huntington's disease (HD) is caused by an expanded trinucleotide stretch of greater than 35 CAG repeats in the *HTT* gene that results in an abnormal polyglutamine tract in huntingtin [1]. HD is phenotypically characterized by progressive motor dysfunction, cognitive impairment, psychiatric symptoms, and personality changes [2, 3]. There is variation in the CAG repeat length mutation among HD patients. Larger CAG repeats are correlated with earlier HD onset [4]. Fully penetrant mutations have 40 or more CAG repeats. CAG repeats lengths of 35 or greater are classified as pathogenic, but mutations with 36 to 39 CAG repeats are incompletely penetrant and often cause later onset HD with slower progression and milder symptoms [5]. Wild type HTT has been shown to have anti-apoptotic effects [6] and plays a critical role in normal neuronal development, transcriptional regulation, cellular trafficking, and synaptic activity [7]. Mutant huntingtin (mHTT) forms intracellular oligomers and aggregates and is thought to have a toxic gain-of-function that interferes with many cellular and biological functions [2]. Previous work using mouse models of HD has shown that mHTT reduction positively influences behavioral and pathological HD symptom development and progression [8, 9]. In the first study of its kind, a regulatory single nucleotide polymorphism (rSNP) in an NF-κB binding site in the *HTT* promoter was

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associated with reduced, allele-specific *HTT* expression that bidirectionally influences HD age of onset [9]. Taken together, these studies support the hypothesis that modulation of *HTT* expression is a viable therapeutic avenue for HD. The huntingtin protein is ubiquitous in both the brain and peripheral tissues [10]. Peripherally, huntingtin is most abundant in the testes and is present in many other tissues, including liver and lung [11]. *HTT* expression patterns within the brain are inconsistent with HD pathophysiology. Expression is highest in the cortex, cerebellum and hippocampus, and is detectable (but not robust) in the striatum [11, 12]. The highest levels of *HTT* transcript and HTT in the brain are found in large neurons and in regions with dense neuronal populations [13]. Regions of reduced HTT within the striatum are positively correlated with regions of neuronal loss in HD brain, indicating that reduced HTT in these areas is the result of neuronal depletion rather than altered *HTT* expression [11, 12].

Various cell-based and animal models of HD have shown that the polyglutamine expansion in mHTT causes accelerated neurodegeneration and neuronal death [14, 15]. Conversely, over-expression of wild type HTT has a modest neuroprotective effect in a variety of model systems. In mice, elevated wild type HTT protects against apoptosis following external toxin exposure [16] and against NMDA receptor-mediated excitotoxicity [17]. In a mouse model of HD, wild type huntingtinover-expression had minimal effect on the HD phenotype, but did reduce the striatal neuronal atrophy caused by mutant huntingtin [18], while depletion of wild type huntingtin levels from conception worsens many aspects of the phenotype in HD mice [6, 19] and increases toxicity in HD cell culture models [20]. In heterozygous HD patients, HTT is expressed from both wild type and mutant alleles [13]. Homozygous HD patients express two mutant alleles in the absence of wild type huntingtin and develop normally with a similar age of HD onset to heterozygotes, but may have a more rapid progression of disease [21]. Finally, the effects of a transcription-lowering rSNP affecting NF-κB binding located in the promoter of the *HTT* gene was associated with a significant delay in age of onset when present on the mutant allele, but the same SNP on the normal *HTT* allele had only a modest effect in accelerating the age of onset [9].Together, these effects suggest that while HD is clearly caused by mHTT expression, the relative proportions of wild type HTT and mHTT may also be important modifying factors in HD.

There are currently are several approaches to post-transcriptional lowering of *HTT* expression in development for the treatment of HD, including small molecules, ASOs, and small interfering RNA (siRNA), as summarized in several recent review articles [22, 23]. Most mHTT-lowering therapies do not distinguish between *HTT* alleles, and studies of HTT-reducing interventions in HD mouse models show clear phenotypic benefits [24]. Characterization of the regulatory mechanisms that control *HTT* gene expression will help expand our understanding of both normal huntingtin function and HD pathogenesis. The manipulation of *HTT* expression has enormous potential as a novel therapeutic approach for HD.

CONSERVED AND UNIQUE HTT PROMOTER REGULATORY ELEMENTS

The *HTT* promoter (Fig. 1) has high GC content and lacks TATA and CCAAT regulatory elements. There is a highly-conserved region between the human *HTT* promoter and the promoter of the mouse homologue, *Hdh.* The sequences in this region, located at positions –206 to –56 relative to the *HTT* translation start site (+1 site), have a 78.81% shared sequence identity. The shared sequence identities of the surrounding regions are lower: approximately 50% [25]. Multiple putative regulatory regions exist within the highly-conserved region. Some elements are shared, while others are specific to the promoter of a single species [25]. Unique features of the *HTT* promoter are: two 20 bp direct repeats, two 17 bp direct repeats (surrounded by identical 7 bp sequences that are identical to the first 7 bp of the full-length 17 bp repeats) and one full *Alu* element and one *Alu* element 3' fragment, both of which are in reverse orientation to the direction of *HTT* transcription (Table 1, Supplementary Figure 1) [25].

One cAMP response element (CRE) in the *Hdh* promoter is absent from the human *HTT* promoter, but is present in the rat homologue, *rhd* [14, 25, 26]. The *rhd* CRE is located within the same region of high conservation shared between *HTT* and *Hdh*. Deletion of this region significantly reduces *rhd* promoter activity in rat neuronal and non-neuronal cells, indicating the presence of regulatory elements necessary for *rhd* expression in this region [26].

A single AP2 binding site is conserved between the *HTT* promoter and the *Hdh* promoter. There are 11 predicted Sp1 binding sites in the *HTT* promoter,

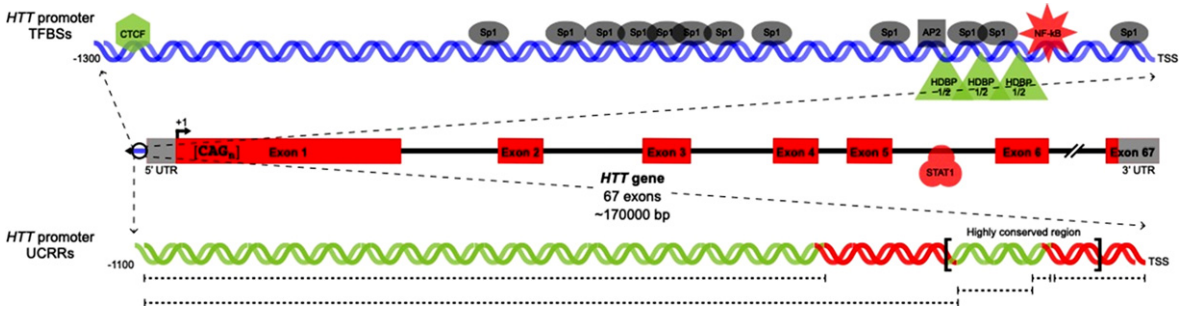

Fig. 1. Previously characterized *HTT* transcription factor binding sites and regulatory regions. Locations of TFBSs in the *HTT* proximal promoter (upper) and gene body (center) are shown. In parallel, uncharacterized regulatory regions in the *HTT* proximal promoter are indicated. TFs/UCRRs shown to upregulate (green) or downregulate (red) *HTT* transcription are pictured in addition to TFs with unknown effects on *HTT* transcription (grey).

and five predicted Sp1 binding sites in the *Hdh* promoter. Only one of these sites is conserved [25]. Four polymorphic sites are present in the region 303 bp upstream of the *HTT* +1 site. One of these sites is predicted to be in the *HTT* 5' untranslated region (UTR). At two of the polymorphic sites, only single base pair substitutions occur. These substitutions are not predicted to be functionally significant because they do not disrupt the *HTT* promoter consensus sequence. The other two polymorphic sites contain one or two copies of a 6 bp repeat sequence or one, two, or three copies of a 20 bp repeat sequence [25, 27]. A single copy of both the 6 bp and 20 bp repeats is present in chimpanzee and gorilla, while the most common human allele contains one 6 bp sequence and a direct repeat of the 20 bp sequence [27].

The differences in regulatory elements between promoters from different species suggest that there may be unique transcriptional regulators for each gene homologue [25]. Conversely, regulatory elements in the *HTT* gene promoter that are shared between species are of interest because their conservation implies functional significance. These gene regions are of high priority for more detailed regulatory characterization.

FUNCTIONALLY SIGNIFICANT HTT PROMOTER REGULATORY ELEMENTS

Specific *in vitro* binding interactions have been identified at one Sp1 site and one AP2 site in the *HTT* promoter [28]. *In vitro* binding interactions also occur between Sp1 and the putative 5' UTR binding site and between Sp1 and the 6 bp polymorphic region. There is no functional effect of duplication of the 6 bp polymorphic region. Deletion of one copy of the 20 bp direct repeat significantly reduces *HTT* expression in both human neuronal and non-neuronal cells. The effect caused by deletion of one copy of the 20 bp direct repeat region shows the Sp1 site located within the 20 bp region is responsible for increased *HTT* expression, and that the observed change in *HTT* expression is not caused by the creation of a novel transcription factor binding site following duplication [28]. In human non-neuronal cells only, deletion of the *HTT* promoter region spanning –126 to –16 causes a decrease in *HTT* expression. In both human neuronal and non-neuronal cells, a total loss of *HTT* expression results from deletion of the region spanning –242 to –171, which is indicative of the presence of one or more positive *cis*-regulatory elements in this region [28].

In vitro binding interactions occur between Sp1 and AP2 at the binding sites conserved between human and mouse, but deletion of these sites has no significant effect on *HTT* expression in both human neuronal and non-neuronal cells [28]. Deletion of the –141 to –126 region causes a reduction in *HTT* expression, which may be the result of either the removal of the 3' transcription start site (TSS) or of an unidentified *cis*-regulatory sequence. Removal of this region causes a more dramatic reduction of expression in human non-neuronal cells than in human neuronal cells [28]. De Souza and Kosior et al. showed siRNA-mediated knockdown of Sp1 in human non-neuronal cells modestly increases *HTT* expression [29]. In both human non-neuronal and neuronal cells, Wang et al. showed that over-expression of Sp1 increases *HTT* expression. Inhibition of Sp1 signaling in the same models decreased *HTT* expression [30]. These contradictory reports mean that the regulatory effect of Sp1 on *HTT* transcription is ambiguous.

Table 1
HTT regulatory elements and features

Regulatory feature/ element	Binding site location relative to *HTT* +1 site	Location relative to +1 site (other species)	*In vitro* binding activity (method)	*In vitro* regulatory activity	Regulatory assay cell type (species)	Effect on *HTT* expression	Element type
20 bp direct repeats[25]	–213 to –194 –193 to –174	None	Not assessed	Yes	Non-neuronal (human) Neuronal (human)	Positive regulator	Predicted*
17 bp direct repeats[25]	–518 to –502 –501 to –485	None	Not assessed	Not assessed	N/A	Unknown	Predicted*
UCRR 1[28]	–126 to –16	N/A	Not assessed	Yes	Non-neuronal (human)	Positive regulator	Predicted*
UCRR 2[28]	–141 to –126	N/A	Not assessed	Yes	Non-neuronal (human) Neuronal (human)	Positive regulator	Predicted*
UCRR 3[28]	–242 to –171	N/A	Not assessed	Yes	Non-neuronal (human) Neuronal (human)	Positive regulator	Predicted*
UCRR 4[31]	–1031 to –221 (w/ HCR)	N/A	Not assessed	Yes	Non-neuronal (hamster) Neuronal (mouse)	Positive regulator	Predicted*
UCRR 5[28]	–1032 to –324 (w/ HCR)	N/A	Not assessed	Yes	Neuronal (human)	Negative regulator	Predicted*
Alu element (full)[25]	–2100 to –1812	None	Not assessed	Not assessed	N/A	Unknown	Predicted*
Alu element (partial)[25]	–1724 to –1598	None	Not assessed	Not assessed	N/A	Unknown	Predicted*
cAMP[26]	None	–180 to –174 (mouse) –175 to –169 (rat)	Yes (EMSA)	Yes	Non-neuronal (rat) Neuronal (rat)	None (Positive regulator of *rhd*)	Putative†
AP2[25,28]	*–249 to –242*	*–270 to –262* (mouse)	Yes (EMSA)	Yes	Non-neuronal (human) Neuronal (human)	None detected	Putative†
Sp1[25,28,29,30]	–15 to –9 –191 to –186 –211 to –206 ***–285 to –279*** –461 to –455 –549 to –543 –579 to –573 –595 to –589 –599 to –593 –646 to –640 –714 to –708 –807 to –813	***–299 to –293 (mouse)*** –318 to –312 (mouse) –374 to –368 (mouse) –379 to –373 (mouse) –427 to –421 (mouse)	Yes (EMSA)	Inconclusive	Non-neuronal (human) Neuronal (human)	Inconclusive	Putative†
HDBP1/2[32]	–221 to –215 –201 to –195 –181 to –175	None	Yes (EMSA)	Yes	Neuronal (human)	Positive regulator	Putative†
p53[29,33,34]	Promoter; intron 2; intron 3	Promoter (partial; mouse)	Yes (ChIP/EMSA)	Inconclusive	Non-neuronal (mouse) Non-neuronal (human) Neuronal (human)	Inconclusive	Putative†
CTCF[35]	–1270 to –1288	None	Yes (EMSA)	Yes	Non-neuronal (human)	Positive regulator	Putative†
NF-κB [9]	–139 to –148	None	Yes (EMSA)	Yes	Non-neuronal (human)	Negative regulator	Putative†
STAT1[29]	*HTT* intron 5	None	Yes (ChIP)	Yes	Non-neuronal (human)	Negative regulator	Putative†

***Predicted** = No binding activity or functional effect of the regulatory element on *HTT* expression has been investigated or detected. †**Putative** = A single group has detected binding activity between the regulatory element and the specified region and observed a functional effect of the regulatory element on *HTT* expression. This classification also applies to sites and elements for which reported binding interactions and/or functional effects have varied between publications. ‡**Independently Validated** = Multiple groups have successfully detected binding activity between the regulatory element and the specified region, and observed a functional effect of the regulatory element on *HTT* expression. **Bolded** site locations indicate conserved sites. *Italicized* site locations indicate sites with experimentally confirmed regulatory element binding activity.

HTT transcription increases when expression of *HTT* is controlled by longer promoter constructs that contain the –1031 to –221 bp region, in addition to the highly-conserved region, upstream from the *HTT* +1 site [31]. Conversely, a decrease in transcriptional activity occurs when *HTT* expression is driven by the promoter containing the highly-conserved region and the –1032 to –324 bp region [28]. These results were reported by separate studies that used, respectively, mouse and human neuroblastoma cell lines to evaluate *HTT* promoter activity [28, 31]. The observed difference in *HTT* promoter activity may be attributed to physiological variation between these cell lines, or may be caused by an unidentified negative *cis*-regulatory element in the –323 to –222 region. Currently, the mechanisms driving the regulatory effects of these *HTT* promoter regions are unknown.

HD gene regulatory region-binding protein (HDBP) 1 and HDBP2 have conserved C-terminal regions that bind to 7 bp sequences separated by 13 bp "spacer" sequences next to the 20 bp direct repeats in the *HTT* promoter [32]. Disruption of the binding sequences destroys HDBP1/2 binding interactions in human neuronal cells. *HTT* promoter function is also reliant upon the preservation of these 7 bp consensus sequences [32]. There is a portion of a putative p53 response element upstream of the *Hdh* +1 site at position –1285. A similar, partial consensus sequence exists in the *HTT* promoter at –630 and –1200 relative to the *HTT* +1 site [33].

Binding interactions between p53 and three regions of the *HTT* gene (in the promoter, intron 2 and intron 3) have been detected *in vitro* with a lower binding affinity than functionally validated p53 response elements at other locations in the genome (for example, within *GADD45*) [34].

In response to γ irradiation-induced DNA damage and resultant p53 pathway activation, an increase in *HTT* expression has been reported in human non-neuronal cells. In murine non-neuronal cells, HTT levels increase following p53 pathway activation by γ irradiation. The recapitulation of this phenotype in neuronal cells has not been reported. The effect of p53 activation on *HTT* expression suggests that p53 is a putative *trans*-activator of *HTT* [34].

There is a positive correlation between levels of p53 and murine htt. An analysis of htt levels in mice with zero, one, or two copies of the mutant *Hdh* allele and zero, one or two copies of the functional p53 allele showed that a larger number of functional p53 alleles increased levels of both wild type and mutant htt [33]. This effect was reported to be most significant in tissue from the brain and testes, and was not observed in peripheral tissues [33]. p53 has the same dose-dependent effect on *HTT* expression in a murine cell model. Transfection of murine fibroblasts with a reporter construct containing an *HTT* promoter fragment driving luciferase gene expression generated a fluorescent signal following p53 over-expression in a dose-dependent manner [33]. In contrast to the Ryan et al. and Feng et al. studies, De Souza and Kosior et al. showed that siRNA-mediated knockdown of p53 in human non-neuronal cells has no effect on *HTT* expression [29]. Currently, it is unclear if p53 is a transcriptional regulator of *HTT*.

Previous analyses of the regulation of *HTT* expression have largely focused on the *HTT* proximal promoter, and overlooked the regions within and surrounding the *HTT* gene. A computational analysis of the *HTT* gene locus (including the entire *HTT* gene, portions the genes immediately upstream and downstream of *HTT,* and intergenic sequences) identified a STAT1 binding site in intron 5 of *HTT* [29].

The *HTT* locus was scanned and scored for putative regulatory markers using publicly available datasets. High scoring regions were probed for putative transcription factor binding sites using ChIP-seq and transcription factor binding profile datasets, and a list of putative *HTT* transcription factors was generated. Analysis of protein-protein interactions and cell type specific DNase hypersensitivity marks prioritized these candidate transcription factors for further investigation. STAT1 was selected for functional characterization [29].

siRNA-mediated knockdown of STAT1 in human non-neuronal cells significantly increased *HTT* expression. STAT1 binding enrichment within *HTT* intron 5 was detected using ChIP-qPCR, confirming the direct interaction of STAT1 with the predicted binding site within *HTT*. The identification of STAT1 as a novel regulator of *HTT* shows that *HTT* transcription can be modulated by regulatory sites outside the proximal promoter region, and within the *HTT* gene body [29].

Epigenetic modifications also contribute to *HTT* expression variability. A CTCF TFBS in the *HTT* promoter has unique methylation occupancy in liver and cortex tissues. siRNA-mediated silencing of *CTCF* reduces *HTT* expression, implicating CTCF as a regulator of *HTT*. Differential methylation of the CTCF binding site may be an additional *HTT* expression modifier that operates in a tissue-specific manner [35].

siRNA-mediated knockdown of NF-κB in human non-neuronal cells significantly increases *HTT* expression [29]. There is a regulatory single nucleotide polymorphism (rSNP) in a NF-κB binding site within the *HTT* promoter. This site changes *HTT* expression by modulating the relative proportions of wild type and mutant HTT. The rSNP impairs NF-κB binding and decreases *HTT* expression. Later age of onset of HD is observed in patients with the rSNP on the mutant *HTT* allele, while a modest effect with an earlier age of onset is observed in patients with the rSNP on the wild type *HTT* allele [9]. The implications of this result are hugely significant, as they show NF-κBbinding to the *HTT* promoter alters *HTT* expression and that this SNP is a bidirectional modifier of HD age of onset. These results are also the first human evidence that modulation of HTT levels alters the course of HD, and supports the development of agents that decrease HTT levels as therapeutic strategies for HD.

OTHER REGULATORS OF HTT EXPRESSION

A recently identified *HTT* antisense transcript that contains the HD CAG repeat tract expansion and has a 5' cap, 3' polyA tail and three exons is detectable in frontal cortex tissues from healthy and HD brains [36]. This antisense transcript is alternatively spliced into two smaller transcripts, one of which contains the CAG repeat tract expansion. An increased number of CAG repeats in *HTT* is correlated with reduced expression of the antisense transcript [36]. Over-expression of the transcript is correlated with decreased endogenous *HTT* mRNA, and siRNA-mediated knockdown of the transcript causes an increase in *HTT* mRNA [36].

CONCLUSION

To date, there have been various attempts to characterize the regulation of *HTT* expression that have yielded inconsistent and often contradictory results. For example, studies have reported different numbers and locations of Sp1 binding sites in the *HTT* promoter [25, 35]. The sequence of the published binding site recognized by Sp1 is a consensus sequence [37], so variability in the query sequence used to predict the locations of Sp1 binding sites may contribute to the inconsistencies between these studies. The same concept can also be applied to functional assays, because transcription factor binding affinities are dependent on the sequences of their binding sites. The relative binding affinities of Sp1 to putative binding sites with different variations of the Sp1 consensus sequence has not been evaluated in the *HTT* promoter. As a result, the actual locations at which Sp1 binding occurs, and the effects of these binding interactions on *HTT* expression, are uncertain.

No studies have successfully reproduced the regulatory effects of transcription factors on *HTT* expression or confirmed the findings reported by other groups. For example, separate investigations have reported that p53 both influences or has no effect on *HTT* expression [33, 34, 36]. According to the criteria we have developed and now propose to classify types of regulatory elements in the *HTT* promoter (Table 1), there are no independently validated regulatory elements that influence *HTT* expression in the literature.

STAT1 is a recently characterized novel regulator of *HTT* expression that binds *HTT* intron 5 [36]. The discovery of this intragenic TFBS indicates that a thorough analysis of *HTT* transcriptional control should probe the entire *HTT* gene locus, rather than focusing only on TF activity at the *HTT* proximal promoter.

There are also no published studies that explore the combinatorial action of transcription factors on the modulation of *HTT* expression. Because many of the regulatory elements previously associated with *HTT* expression are nonspecific transcription factors, it is possible that the specificity of *HTT* expression is conferred by the synergistic action of these factors. This type of regulation may also explain why some studies report no functional effect of transcription factor binding site disruption, even though binding interactions between transcription factors and these sites have been validated using *in vitro* methods in the same studies.

More generally, inconsistencies in the literature may also be attributed to the methods of investigation used(for example, cell type) or to the length of the regulatory regions examined. Regardless of the source of the variability, it is apparent that, to date, no comprehensive evaluation of the regulation of *HTT* expression has yet been conducted. A thorough, functionally-validated assessment of the regulators of *HTT* expression, and how *HTT* expression changes in response to external stimuli, will be important for the continued development of novel therapeutic strategies aimed at reducing mHTT levels.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JHD-180331.

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