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Neuronal Ca^{2+} dyshomeostasis in Huntington disease

Marta Giacomello,[1,*] Juan C. Oliveros,[2] Jose R. Naranjo[2,3] and Ernesto Carafoli[4,*]

[1]University of Geneva; Geneva, Switzerland; [2]Centro Nacional de Biotecnología; Consejo Superior de Investigaciones Científicas (CSIC); Madrid, Spain; [3]Centro Investigacion Biomedica En Red de Enfermedades Neurodegenerativas (CIBERNED); Madrid, Spain; [4]Venetian Institute of Molecular Medicine; Padova, Italy

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The expansion of the N-terminal poly-glutamine tract of the huntingtin (Htt) protein is responsible for Huntington disease (HD). A large number of studies have explored the neuronal phenotype of HD, but the molecular aethiology of the disease is still very poorly understood. This has hampered the development of an appropriate therapeutical strategy to at least alleviate its symptoms. In this short review, we have focused our attention on the alteration of a specific cellular mechanism common to all HD models, either genetic or induced by treatment with 3-NPA, i.e., the cellular dyshomeostasis of Ca^{2+}. We have highlighted the direct and indirect (i.e., transcriptionally mediated) effects of mutated Htt on the maintenance of the intracellular Ca^{2+} balance, the correct modulation of which is fundamental to cell survival and the disturbance of which plays a key role in the death of the cell.

Introduction

All patients affected by Huntington disease (HD) have as a common genetic defect, the expansion in the number of CAG triplets located in the N-terminal region of the protein huntingtin (Htt). Normally, the wild type protein is characterized by 15–35 CAG repeats, while in the Htt forms associated with HD the repeats increase up to 40–180. One of the hallmarks of HD is the presence, within the cell body, of insoluble inclusions, composed of aggregates of Htt fragments, produced by the cleavage of the protein by caspases, characterized by the trapping of very diverse proteins, among them calmodulin, transcription factors, components of the ubiquitin-proteasome system and polyubiquitin binding proteins, e.g., p62 that is involved in autophagy.[1,2]

Even if Htt is ubiquitously expressed, HD is characterized principally by specific motor and cognitive impairments, suggesting a precise role of Htt in certain tissues and brain domains. The exact function of Htt has not been clarified; however, many tasks for it have been proposed based on the results obtained in different models of HD that have highlighted impairment in organelle and vesicular trafficking, cholesterol biosynthesis and propensity to apoptosis.[3,4] Numerous papers have also reported an imbalance in the generation and scavenging of reactive oxygen species (ROS),[5,6] as well as defects in the respiratory chain complexes[7,8] and in mitochondrial functions and morphology.[9] A detailed discussion of these specific alterations, which have been already summarized elsewhere,[4,10] is beyond the scope of this review, which will focus on the changes of intracellular Ca^{2+} homeostasis induced by mutated Htt.

The concentration of Ca^{2+} ($[Ca^{2+}]$) within the cell is finely tuned by a series of mechanisms, since Ca^{2+} is a messenger that modulates different signal transduction pathways that are vital to cells: their disturbance can eventually even lead to cell death. Among the main actors involved in $[Ca^{2+}]$ handling there are proteins that act as Ca^{2+} buffers, proteins that export Ca^{2+} from the cytosol toward the extracellular medium (the plasma membrane Ca^{2+} pumps and Na^{+}/Ca^{2+} exchangers) or to the lumen of organelles and proteins that mediate Ca^{2+} entry in the cytoplasm: the influx of Ca^{2+} is mediated by a number of Ca^{2+} channels (among them those formed by the STIM/Orai proteins which are involved in the store operated Ca^{2+} entry (SOCE) process). Finally, there are proteins whose function is activated by binding to Ca^{2+}, such as DREAM and calmodulin.[11,12] The membrane of some organelles, e.g., that of mitochondria and the endoplasmic reticulum (ER), also contain systems that take up/extrude Ca^{2+}, establishing a Ca^{2+}-linked crosstalk with neighboring organelles.[13,14]

Work on various animal models in which HD was either induced by the genetically produced presence of mutated Htt or by the treatment with 3-nitropropionic acid (3-NPA), an inhibitor of a component of the mitochondrial respiratory chain, complex II, that has been shown to induce a HD phenotype,[15] has indeed suggested that one of the hallmarks of HD is the impairment of the intracellular $[Ca^{2+}]$ modulation. The presence of mutated Htt fragments has been associated to the altered expression of some genes involved in Ca^{2+} homeostasis both in human patients and in HD murine models.[4,17] Direct binding of mutated Htt fragments to proteins involved in Ca^{2+} handling has also been reported.[16]

Both effects have been supported by convincing evidence. However, it is still unclear whether the transcriptional effects in HD neurons are a cell adaptation response to the variations of intracellular $[Ca^{2+}]$, which could be due to the direct interaction of mutated Htt (and/or Htt fragments) with Ca^{2+} binding/Ca^{2+} transport proteins, or whether the changes of Ca^{2+} levels are instead a secondary consequence of primary effects of mutated

*Correspondence to: Marta Giacomello and Ernesto Carafoli;
Email: marta.giacomello@unige.ch and ernesto.carafoli@unipd.it
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Htt on the expression of regulatory proteins. It is of course still possible that transcriptional and non-transcriptional processes run in parallel and contribute jointly to the onset of HD.

Intracellular Ca^{2+} Dyshomeostasis in HD

Defects of intracellular Ca^{2+} homeostasis have been reported in the majority, if not all, the experimental models of HD. However, different alterations have been described depending on the model used. For instance, in medium spiny neurons from mice expressing exon 1 of the human HD gene (R6/2 mice) an increase in the basal cytosolic [Ca^{2+}] has been detected that could induce an adaptation to excitotoxic stress.[18] In clonal striatal progenitor cell lines obtained from knock-in mouse embryos carrying a 111 poly-glutamine repeat, we have reported an increased release of Ca^{2+} from the ER induced by the blockade of the SERCA pump with cyclopiazonic acid with respect to the wild type controls.[9] Defects in the morphology of mitochondria have also been observed in the same cell line. The organelles appeared more fragmented: the deregulation of cytosolic Ca^{2+} contributed to the upregulation of the activity of the Ca^{2+}-dependent phosphatase calcineurin that could dephosphorylate (and thus activate) the pro-fission dynamin related protein 1 (Drp1) leading to the fragmentation of mitochondria and increased propensity to apoptosis.[9]

Interestingly, the majority of the papers on Ca^{2+} dyshomeostasis in HD describe principally defects on Ca^{2+} uptake/extrusion by mitochondria, which are known to act as central regulators of intracellular Ca^{2+} levels. When the concentration of cytosolic [Ca^{2+}] in the vicinity of mitochondria increases significantly, the low-affinity mitochondrial Ca^{2+} uniporter (MCU) becomes activated and mediates Ca^{2+} entry into the matrix. Mitochondria release Ca^{2+} by means of a Na^{+}/Ca^{2+} exchanger[13] or, in case of Ca^{2+} overload, through the opening of the permeability transition pore (PTP),[14] which results in collapse of the membrane potential, rupture of the outer membrane and release of cytochrome c.[14] Alterations in the Ca^{2+} buffering/handling activities of mitochondria have been reported in both early and late stages of HD, indicating a key role of these defects in its pathogenesis. Mitochondria isolated from cells obtained both from HD patients and HD murine models (carrying 72 or 150 poly-glutamine repeats) are more sensitive to Ca^{2+} loads, i.e., they have a lower [Ca^{2+}] threshold for the depolarization of their inner membrane and higher propensity to PTP opening.[19,20] Similar mitochondrial Ca^{2+} handling defects have been also reported in clonal striatal cells expressing an Htt form carrying 111Q.[21]

Results of this type could easily explain the higher susceptibility of HD cells to apoptosis; however, they have not been reproduced in all HD experimental models. For instance, striatal mitochondria from knock-in mouse models expressing different variants of Htt (with 20, 80, 92 or 111 glutamine stretches), from R6/2 mice (3 months old) and from 12 months YAC128 mice (carrying a mutated Htt with 128 CAG triplets) were equally or even less susceptible to Ca^{2+} loads than their wild type counterparts.[22,23] In addition, the sensitivity to Ca^{2+} of mitochondria from several HD models decreased in parallel with age and polyQ length,[22] suggesting the intervention of compensatory mechanisms that would eventually protect these organelles from PTP opening.

In addition to interfering with mitochondrial Ca^{2+} handling, Htt also influences the release of Ca^{2+} from the ER and the function of SOCE channels. As to the ER, mutated Htt (but not the wild type protein) has been claimed to interact directly with the C-terminal portion of the inositol 1,4,5-trisphosphate ($InsP_3$) receptor type 1 ($InsP_3R1$),[16] sensitizing it to $InsP_3$[24] and thus modulating the efflux of Ca^{2+} from the ER. As suggested for other neurological disorders,[25] this could in turn affect the ability of mitochondria to take up Ca^{2+}, that is known to depend on the close proximity of mitochondria to the Ca^{2+} releasing ER. A recent finding has shown that InsP_3R silencing reduces the aggregation of mutated Htt,[26] underlining the importance of the interaction between the two proteins for the molecular pathogenesis of HD.

Interestingly, the association of Htt fragments with ER membranes has been recently confirmed and reported to be dependent on ER stress:[27] the translocation of Htt from the ER to the nucleus was induced by ER stress. Both the binding to the ER and the shuttling of the Htt fragments to the nucleus were affected by the length of the glutamine repeat.[26,28,29]

The capacitative, SOCE channels mediated entry of Ca^{2+} from the extracellular medium also appears to be affected in HD. Convincing experimental evidence has indeed shown that this process is enhanced in HD cells.[30] Inhibitors of the capacitative Ca^{2+} entry slow down the progression/appearance of HD phenotypes in transgenic flies and exert protective effects on primary cultures of medium spiny neurons from YAC128 mice.[30] SOCE channels could thus be a possible novel target for the design of new drugs to alleviate the symptoms of HD.

Full length and N-terminal fragments of Htt have also been reported to bind the synaptic region of N-type voltage dependent Ca^{2+} channels,[31] possibly modulating their activity. Finally, mutated Htt can directly interact with numerous Ca^{2+} binding proteins.[32] This ability should not be underestimated, since, as mentioned above, mutated Htt could sequester these proteins, preventing them from fulfilling their normal physiological tasks and leading to the impairment of signal transduction pathways.[33] Notably, mutated Htt fragments have been found associated to Calmodulin in high molecular weight complexes,[32] disruption of this interaction exerting neuroprotective effects.[34,35] Another of the sequestered proteins is Calretinin, a calcium-binding protein with six EF-hand Ca^{2+} binding domains: the interaction between Calretinin and mutated Htt seems to have protective effects since Calretinin knockdown increases the susceptibility of HD cells to cell death, while its overexpression alleviates the [Ca^{2+}] alterations associated to the presence of mutated Htt.[36]

One of the hallmarks of mutated Htt is its ability to aggregate, thus the question raises on whether Ca^{2+} dyshomeostasis is playing a role in the aggregation process or, in turn, whether the presence of Htt inclusions correlate with the appearance of Ca^{2+} deregulations. Actually the latter has been investigated in detail in a very elegant paper in which the authors overexpress in PC12 cells a truncated form of Htt (Httex1p) containing the polyglutamine repeat.[37] Overexpression of Httex1p containing

Table 1. Transcriptomic experiments compared in the bioinformatics analysis

GEO ID	Organism (GeneChip)	Experimental (replicates)	Control (replicates)
GSE3583	*Mus musculus* (MG430v2.0)	Wild type Q7 cells 3NP treated (3)	Wild type Q7 cells untreated (3)
		Mutant Q111 cells (3)	Wild Type Q7 cells (3)
GSE18551	*Mus musculus* (MG430v2.0)	YAC128 mice 12 mo old (4)	Wild type mice 12 mo old (4)
		YAC128 mice 24 mo old (6)	Wild type mice 24 mo old (4)
GSE1751	*Homo sapiens* (HGU133A)	HD symptomatic (12)	Healthy controls (14)
		Presymptomatic (5)	Healthy controls (14)

The Gene Expression Omnibus (NCBI-GEO, www.ncbi.nlm.nih.gov/geo/) ID of each experiment is shown on the right. In each case, the species and the experimental vs. the appropriate control are indicated. YAC128 is a transgenic mouse model for HD expressing a yeast artificial chromosome containing the mutated Huntingtin gene with 128 CAG repeats. Q7 and Q111 cells are neuroblasts cells derived from striatal neurons obtained from wild type or mHtt knock-in mice, respectively.

97 glutamines caused both an alteration of the basal Ca^{2+} levels as well as an increase in the intracellular Ca^{2+} levels induced by glutamate. On the contrary, expression of the wild type Httex1p caused no variations in the resting Ca^{2+} levels, suggesting a key role for the expansion of the glutamine repeat on Ca^{2+} dyshomeostasis. As to the effect of aggregates per se on Ca^{2+} dyshomeostasis, it is worth mentioning that expression of mutants forms of Httex1p carrying either 97 or 103 glutamines deprived of the first 17 amino acids (thus causing a reduction of their ability to aggregate) was sufficient to alter the basal Ca^{2+} levels, but not the response to glutamate stimulation.

Transcriptional Deregulation of Ca^{2+} Handling Proteins in HD

Transcriptional deregulation has been described in almost every model of HD, suggesting a fundamental role for it in the molecular pathogenesis of the disease.[38,39] The transcriptional effects have been ascribed to a variety of mechanisms: impairment of microRNA mediated gene expression,[40,41] direct binding to the DNA and interaction with transcription factors both inside the nucleus and within the cytoplasm.[39,42]

The preferred mechanism seems to be direct binding: Htt fragments could behave like a "kidnapper," sequestering negative and positive regulators of transcription in the Htt inclusion bodies. Among the molecules sequestered a number are indeed factors that regulate gene expression, some of which also contain glutamine stretches. This is the case of the CREB-binding protein (CBP),[43] a coactivator of CREB (cAMP-responsive element binding protein). Interestingly, transient overexpression of CBP has protective role in cellular models expressing mutated Htt,[43] and disruption of CREB function leads to a striatal phenotype reminiscent of HD.[44] The impairment of CBP activity could have particularly detrimental consequences given its function as a master regulator of the peroxisome proliferator- activated receptor γ (PPARγ) coactivator-1a (PGC1α). PGC1α is primarily involved in mitochondria biogenesis: thus, impairment of its expression could contribute to the production of the mitochondria defects described above. The possible role of the impairment of PGC1α expression on the onset and progression of HD is supported by experimental evidence; the majority of HD models present changes in the level of PGC1α, and its overexpression has protective effects on striatal neurons expressing mutated Htt.[44] Finally, PGC1α Knock out mice present lesions in the striatum and phenotype similarities with HD murine models.[45,46]

The genes that show transcriptional deregulation in HD are involved in numerous physiological processes. They range from those coding for molecules that act as neurotrophins [this is the case of the brain-derived neurotrophic factor (BDNF), whose mRNA levels are significantly lower in human and murine HD samples,[47,48] for protein kinases (such as protein kinase C β II[17])] or for cytosketetal proteins.[17] Importantly, many genome-wide studies performed on various HD models have shown profound differences in the mRNA levels of genes coding for Ca^{2+} binding proteins, i.e., parvalbumin, calbindin or hippocalcin or proteins involved in the regulation of intracellular Ca^{2+} handling, i.e, the ryanodine receptor type 1 or different voltage-dependent plasma membrane Ca^{2+} channels.[17,38] To better illustrate this point, we have performed a simple bioinformatics analysis using results archived in public databases. Thus, several public gene expression studies performed with Affymetrix genechips (**Table 1**) were combined in one single file of logRatios for approximately 650 probes corresponding to genes related to Ca^{2+} homeostasis in neurons according to Affymetrix annotations to probesets included in their genechips. Four representative HD model systems were selected, the mouse Q7/Q111 knock-in striatal neurons, Q7 (wild type) neurons exposed to the mitochondrial toxin 3-NPA, the striatum from the YAC128 transgenic mice and lymphocytes from HD patients or pre-symptomatic mHtt carriers with their appropriate healthy controls (**Table 1**). A total of six individual genechip hybridization experiments were included in the analysis (**Table 1**).

Prior to performing the bioinformatic analysis, to allow the comparison between human and mouse genechips, all probeset IDs from the human genechip (HGU133A) were converted to the orthologous probesets for the mouse Affymetrix GeneChip (MG430v2.0), by using the conversion tables provided by Affymetrix (www.affymetrix.com). Normalized log2Intensity values provided by GEO database (www.ncbi.nlm.nih.gov/geo) were averaged over all replicates (AvgLogIntensities). For each comparison LogRatios were calculated as: LogRatio = AvgLogIntensity (experiment) – AvgLogIntensity (control). Twenty-five clusters of similar logRatio patterns were generated by K-means algorithm (**Fig. 1**). Some of the most significant clusters from **Figure 1** are commented below.

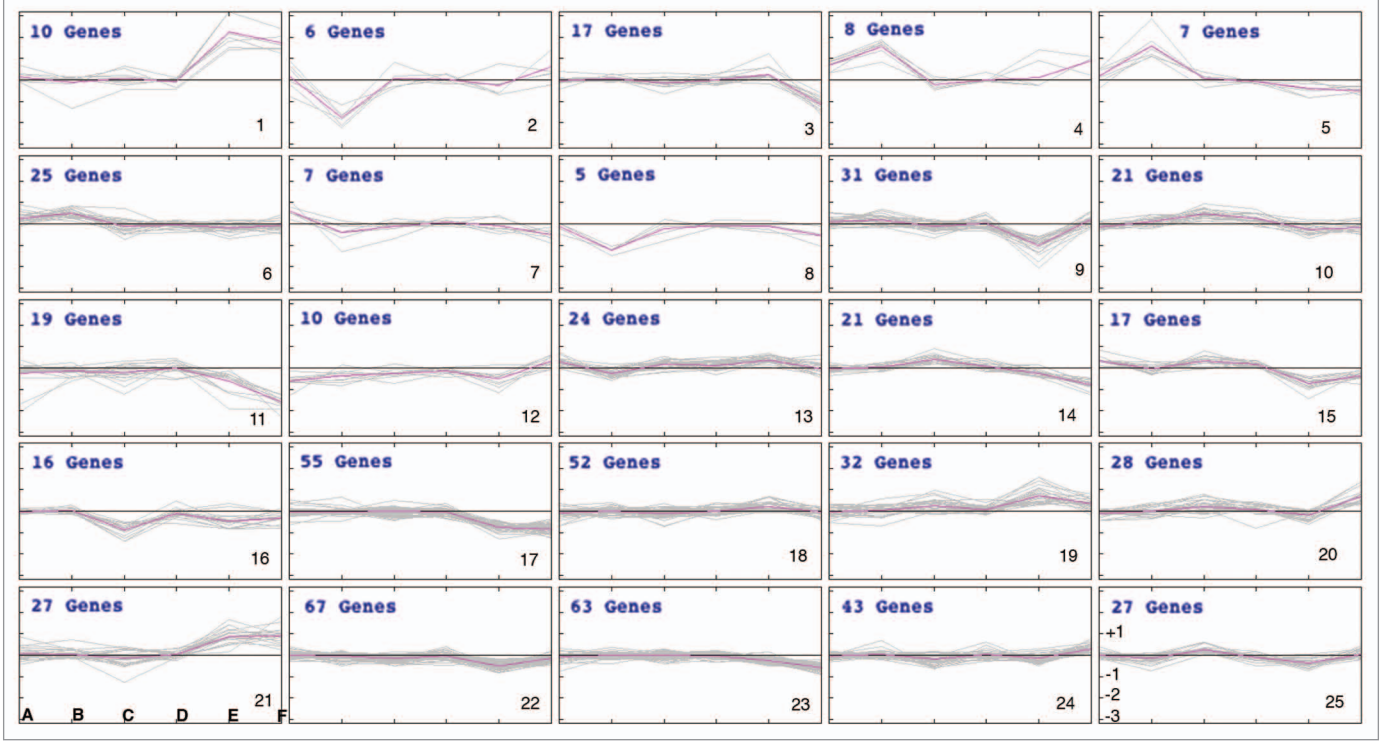

Figure 1. Representation of the 25 clusters of similar logRatio patterns generated by K-means algorithm. LogRatios were calculated as described, from the normalized intensities downloaded from GEO database for each experiment. They represent differential expresión of the genes in the altered samples (disease or transgenic) vs. the controls (healthy or wild type). LogRatios (from -3 to +3) are represented in the Y-axis in each cluster, as shown for cluster 25 at the bottom right corner. LogRatio equal to 0 is represented by the horizontal middle line in each cluster. Positive logRatios (above the horizontal middle line) indicate overexpression whereas negative logRatios (below the horizontal middle line) indicates under-expression. Clusters 1 to 25 are numbered in the bottom right corner. For each cluster the six experiments are labeled (**A–F**) (as shown for cluster 21, bottom left corner). (**A**) Q7–3NP vs. Q7; (**B**) Q111 vs. Q7; (**C**) YAC128-12 min vs. WT-12 min; (**D**) YAC128-24 min vs. WT-24 min; (**E**) HD vs. Normal and (**F**) Pre-symptomatic HD vs. Normal. Each cluster represents the coordinated changes in gene expression of a subset of calcium-related genes for each of the six experimental conditions (**A–F**) compared in this study.

Genes related to calcium homeostasis that are downregulated in lymphocytes from HD patients, Yac128 mice and Q111 knock in cells (clusters 22 and 11) are shown in **Figure 2** and in supplemental **Figure 1A**, respectively, while genes that are downregulated only in HD patients (cluster 17) or in HD patients and YAC128 mice (cluster 16) are shown in **Figure S1B and C**, respectively. Similarly, cluster 19 includes the most representative collection of genes related to calcium homeostasis that are induced in all four model-systems of HD (**Fig. 3**), while others in clusters 21 and 1 are mostly induced in human HD lymphocytes (**Fig. S1A and B**). Moreover, we have found that another significant number of genes change in opposite directions in the different model systems compared in this analysis. Thus, several genes included in clusters 10 and 15 are downregulated in human samples but are induced in non-human models (**Fig. 4A and B**). In summary, this preliminary bioinformatics analysis shows coordinated or divergent changes in the expression rate of many calcium homeostasis-related genes, that involve a wide variety of cellular functions related to calcium signaling, in HD. Several factors, however, preclude a more detailed commentary for each set of genes at this stage. For instance, the number of experiments introduced in the comparison is reduced specially when compared with the diversity of the experimental specimens that are compared. Thus, differences observed in the behavior of certain sets of genes could be related to the different tissues subjected to genome-wide analysis, human lymphocytes vs. neuronal tissue or cells or to the different approaches used to generate the HD model, i.e., the generally poor agreement between the transcriptional changes observed in striatal Q111 neurons and those present in Q7 cells after exposure to the 3-NPA toxin (**Figs. 2–4**). Further comparative bioinformatic analyses including more genome-wide studies would be required to complete the assessment of changes in calcium homeostasis in HD that are controlled at the transcriptional level.

The transcriptional defects in the expression of proteins involved in the maintenance of intracellular Ca^{2+} homeostasis has certainly a role in the Ca^{2+} dyshomeostasis observed in HD models. The transcriptional defects could be further amplified by Ca^{2+} dependent transcriptional control mechanisms. This could occur by changes in the function of gene expression regulatory proteins modulated by Ca^{2+} binding. This is the case of the transcriptional repressor DREAM [downstream responsive element (DRE) antagonist modulator], that translocates to the nucleus following the increase of cytosolic [Ca^{2+}] levels,[49,50] or of the cofactor LMO4 (LIM domain only 4), the activity of which is induced by Ca^{2+} entry through voltage dependent Ca^{2+} channels.[51]

Figure 2. For figure legend, see page 81.

Figure 2 (See opposite page). List of the 67 Ca^{2+}-related genes in cluster 22 that are downregulated in the model systems of HD shown on the top. Color-coded LogRatio from -3 to +3 is shown on the top. For each gene in the cluster the acronym and the full name is shown. MultiExperiment Viewer software[54] was used for generating and plotting the clusters.

Figure 3. List of the 32 Ca^{2+}-related genes in cluster 19 that are upregulated in the model systems of HD shown on the top.

Conclusions

This short review has succintly summarized the present knowledge on the intracellular Ca^{2+} alterations that occur in HD models. Mutated Htt can directly bind to the InsP_3R, thus modulating the ability of its channel to release Ca^{2+} and thus in turn the crosstalk between the ER and mitochondria and the active mitochondrial Ca^{2+} uptake.[16,52,53] This could be a general mechanism in many neuropathological processes, since disturbances in the formation of peri-mitochondrial Ca^{2+} microdomains has been already reported to contribute to other disorders, such as for example Alzheimer disease.[25]

In parallel, strong evidence underlines the importance of transcriptional dysregulation in HD and of the ability of mutated Htt fragments to sequester or modulate proteins involved in gene expression. Interestingly, this is reflected in the changes of the expression of numerous genes related to Ca^{2+} homeostasis,[17,38] raising the question of whether these two key processes that are affected in the disease run in parallel or in series.

A conciliatory hypothesis on the transcriptional and non-transcriptional effects of mutated Htt could be suggested by the interesting finding of Htt translocation from the ER to the nucleus.[27-29] The expansion of the glutamine repeat in mutated Htt could affect the nuclear shuttling of Htt itself. At the same time it could cause the formation of inclusion bodies where transcription factors could be sequestered, in turn again influencing the expression of various genes, among them some coding for proteins of the Ca^{2+} homeostasis machinery.

Figure 4 (See opposite page). Lists of the 21 and 17 Ca^{2+}-related genes in clusters 10 (**A**) and 15 (**B**), respectively, that are differentially regulated in the different model systems of HD shown on the top.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here:
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