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EC NEUROLOGY
Review Article

Huntington's Disease and Nuclear Medicine

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Received: May 23, 2025; **Published:** June 10, 2025

Abstract

PET studies of Huntington's Disease (HD) have been used to monitor receptor binding and transport, oxygen utilization, and cell signaling related to synaptic loss and cell death. This focus has left earlier molecular events that precipitate neuronal cell loss poorly identified. Given the monogenetic origin of HD, however, there is increasing recognition that therapeutic intervention will need to be based on a knowledge of these early events, particularly that of the mutant huntingtin protein (mHTT). Studies of the normal variant (HTT) are providing insight into the protein's structure, genetic expression, various cellular processes such as transport and autophagy, and interactions between huntingtin and its protein partners, illuminating the potential early role of mHTT in HD dysfunction.

Advances in PET instrumentation and radioligand procedures are helping to bridge this molecular scale domain with the macroscale domain of brain communication. Driving these advances is the search for multi-information data sources that can be precisely targeted, which has yielded multimodal sensing technology and precision guided probes like reporter proteins and immunoconjugates. This chapter reviews current developments in nuclear medicine that seek to address early-stage HD dysfunction.

Keywords: *Huntington's Disease; Positron Emission Tomography; PET/MRI; Huntingtin; Radiogenomics; Intrabodies; Reporter Genes; Anti-Sense Oligonucleotides; fMRI; PET/MRS*

Introduction

Huntington's disease (HD) is a terminal, neurodegenerative disorder lasting between 10 to 30 years from symptom onset [1]. Although variable, its symptoms display a characteristic triad of motor, cognitive, and psychiatric features that increase in severity as the disease progresses. During the premanifest phase, subtle changes in motor control, cognition, and personality can be observed, which later evolve to manifest HD symptoms [2]. The disease's chief neurological features relate to a dominant striatal cell loss of medium spiny neurons, with some degeneration of neurons also occurring in the cerebral cortex [3].

Genetically, HD is an autosomal dominant disorder, having a mean age at onset of 40 years [1] and a prevalence of about 5 - 10 individuals per 100,000 among European descendants. The causal basis of the disorder is known to involve a mutation in the huntingtin gene that encodes a polymorphic polyglutamine (polyQ) stretch in the N terminal segment of the huntingtin gene product. Since its identification as the causal factor in HD, the gene product has become increasingly well characterized. It is a large, 348 kDa protein composed of 67 exons, containing various specialized sites for proteolysis, modulation, and binding [4], which participate in its various

functions. While the polyQ stretch is apparently the causal factor within the protein leading to the disease's neurodegenerative features, it is the remainder of the protein that likely confers the unique degenerative features that mark the disease [4].

Increasing knowledge of the protein's molecular features and its manner of participation in cellular functions is contributing to improved understanding of how the mutant protein affects the initial cellular events leading to neurodegeneration. It is known, for example, that the protein engages an array of functions, including cellular transport and dynamics, gene expression, and autophagy [5]. However, the range of functions is not all encompassing, suggesting that impairment of a select suite of key functions may be chief factors leading to neuronal loss. Indeed, unlike other neurodegenerative diseases like Alzheimer's, for which causal factors have yet to be identified, the identification of a causal factor with the huntingtin gene product has enabled the clarification of the molecular processes likely to be affected by the mutant product [4].

PET imaging has played a pivotal part in the traditional understanding and diagnosis of HD [6,7]. PET's non- invasive molecular imaging technique has notably been used to investigate glucose metabolism, the dopaminergic system, phosphodiesterase activity, and neuroinflammation [8], with PET procedures often used to monitor neurotransmitter and neurotransmission loss as a proxy for neural dysfunction. PET imaging of dopaminergic terminals, for example, is used as an indicator of neural dysfunction in the striatum. Such studies have been useful in assessing the pathophysiological state of the disease, where neuronal dysfunction and/or death was well advanced. On the other hand, imaging of neurotransmitter dynamics such as dopamine is not specific to HD pathology and dopaminergic medications have generally yielded inconclusive outcomes for HD patients. Hence, there is a clinical need to use improved PET methods for assessing early (pre-manifest and mHTT induced) events that lead to loss of functional connectivity and degeneration. The ability of PET imaging to assess these dynamics has the potential to yield more precise diagnoses about the state of the disease [6,8,9] before manifest, degenerative symptoms occur.

The rapidly growing body of knowledge about the protein and its cellular functions is helping to illuminate a much broader role for which nuclear imaging techniques could be employed for HD diagnosis and therapy [8,9]. Given the ability of nuclear imaging to detect subtle alterations at the nanomolecular level, a prerequisite for assessing minimal changes in affected brain cells, nuclear medicine procedures are poised to provide precise information about early molecular events in HD. Key to these objectives has been the ongoing technological evolution in PET imaging, together with new methods for the targeting and delivery of radiolabeled probes [10]. Emphasis on quantitation and information diversity, for example, has led to the development of multimodal and dual probe systems capable of simultaneously comparing a suite of parameters as they change in time [11,12]. In one such development multi- dimensional information recovery has driven the emergence of radiogenomics [13].

In this method magnetic resonance spectroscopy (MRS) and PET imaging data are correlated with data from genome sequencing to yield correlation matrices used to guide personalized treatments in the absence of pathologic specimens. Other developments combine functional MRI methods with PET imaging to relate molecular dynamics to brain network connectivities.

The advances in technology are being coupled with a growing trend in radiolabeling design that emphasizes targeted delivery and molecular selectivity. Radionuclide imaging studies in the brain, particularly those related to gene therapy, must address challenges of crossing the blood brain barrier (BBB) and the delivery of probes to select sites. This need has led to vehicles for crossing the BBB that include both specialized nanoparticles and a host of viral vectors that can be loaded with radiolabeled probe, targeted to select cell classes [14,15]. Additionally, an emerging class of methods employs gene editing methods to incorporate 'reporter' genes into cell genomes, which then express gene products that are functionally and location specific [16]. Reporter gene imaging is developing rapidly due to the need to monitor cell and gene therapies by providing critical information on the biodistributions, magnitudes, and durations of the

imaged gene product [10]. Still another class of procedures employs antibodies or antibody fragments as radioligands that can target select protein sites critical to subcellular processes [9], thereby functioning as theranostics to both suppress dysfunctional interactions and monitor the effectiveness of such therapy.

This chapter will focus on emerging developments in PET technology and probe design that have clinical relevance for early dysfunctional changes in HD.

Molecular Events Affected by Mutant Huntingtin

Studies of the HTT gene and gene product are informative for understanding how the gene mutation may lead to loss of neural communication and neural degeneration [4,9]. For example, studies of proteins known to interact with HTT has revealed that HTT participates in multiple cellular functions that are disrupted by the mutation suggesting their likely contribution to neuronal loss. This section focuses on recent discoveries surrounding the protein and its cellular roles, a prerequisite for assessing their relevance for use in nuclear medicine.

The Huntingtin protein

The HTT gene encodes a large, 348 kDA protein that is well conserved across a broad species range. The protein's N-terminal region contains the CAG repeat that codes for the variable, polyglutamine (polyQ) stretch. Whereas in normal individuals this sequence can be repeated up to 35 times, exceeding this length has been shown to correlate with the appearance of HD symptoms, with disease severity and age of onset proportional and inversely proportional to increasing length, respectively. While significant, the polyQ expansion is itself relatively non-specific, inducing eight other known neurodegenerative disorders [4]. Since each disease is associated with a specific difference in another region of the protein that is correlated with the loss of specific neurons, the unique symptoms of HD appear to require features unique to domains of the huntingtin protein outside the N-terminal region.

The N-terminal 17 amino acid segment [4] precedes the polyQ stretch and is important for retention in the endoplasmic reticulum, where it functions as a nuclear export signal. This segment includes a number of sites at which post-translational modifications can occur, including acetylation, sumoylation, and ubiquitination (lysines 6, 9, and 15) and phosphorylation (serines 13 and 16), modifications that affect the protein's clearance and subcellular localization.

Several HEAT repeat domains outside this region afford potential scaffolding sites, which are posited to engage in multiple inter- and intra-molecular interactions. Consistent with this, purified HTT can assume more than 100 structurally distinguishable conformations and is known to interact with a wide spectrum of protein partners, with affinity column assays identifying as many as 234 proteins interacting with HTT [4].

Gene expression and processing

Cellular processes potentially altered by mHTT include those affecting gene expression and processing. Consistent with a transcriptional role, HTT is present in the nucleus, as well as being present in the cytoplasm [4]. The protein is also known to contain a polyQ tract, which acts in the nucleus as a transcriptional regulating domain. Further, wild-type HTT binds numerous transcription factors, including the cAMP-response element (CREB)-binding protein (CBP), NeuroD, the specificity protein-1 (SP1), the nuclear factor-kB (NF-kB), and the tumor suppressor protein 53 (p53) [17]. Through these interactions, HTT could potentiate or repress transcription thereby affecting many cellular processes. For instance, due to p53 interactions with HTT [18], HTT likely influences the transcription of p53 target genes, which are involved in cell-cycle control, apoptosis, cellular stress responses, and DNA repair.

Besides observations of transcriptional dysregulation in postmortem human HD brains and mouse models, extensive changes in histone acetylation levels have been observed in cellular systems in which mutant HTT is overexpressed [19]. The changes occurred

at select loci, which included promoters of such down-regulated genes as Drd2, Penk1, Actb, or Grin1. The reductions also occurred in enhancer regions known to contain super-enhancers, that is regions which regulate genes specifying cell type identity, plasticity, and function, and were seen in the striatum, a region dramatically affected in HD. These results suggest that down regulation of genes defining neuronal identity and function feature prominently in HD genetic expression.

Clearance and proteolysis

HTT undergoes proteolysis at several sites, including amino acids proline (P), glutamic acid (E) or aspartic acid (D), serine (S), and threonine (T) [20], which involve the proteases calpain, caspases, cathepsins, and the metalloproteinase MMP10. Depending on the type and siting of proteolysis in normal HTT, various cellular responses are initiated. For example, cleavage of HTT at positions 586 and 552 yields a 553–586 fragment that, when myristoylated, leads to autophagosome formation [4,20].

In line with this, evidence for an mHTT associated impairment in protein processing and autophagy has emerged in studies highlighting autophagy impairment. The activity of these proteolytic enzymes specifically increases in brains of HD patients and leads to the generation and accumulation of small N-terminal fragments that can translocate into the nucleus where they are toxic. PolyQ-HTT has been shown to abnormally activate autophagy in HD models via inactivation of the rapamycin (mTOR) kinase target [21]. It has also been shown that HTT binds to p62, which promotes recognition of ubiquitinylated proteins at Lys 63 by p62, a process that facilitates cargo loading into autophagosomes. Failure of one, or both, of these mechanisms is thought to result in a defect in autophagosome loading, thereby diminishing the capacity of neurons to degrade protein complexes and organelles [4].

Cellular transport and molecular partners

Consistent with HTT acting as a scaffold hub - binding studies show that HTT may interact with well over a hundred different proteins [4] - the protein is capable of tethering partners into multiprotein complexes. In the case of cellular transport, HTT interacts either directly with dynein or via the Huntingtin-associated protein 1 (HAP1) with the p150*Glued* subunit of dynactin and the kinesin 1 member KIF5C [9], a process modulated by phosphorylation. By means of these interactions, HTT can transport a variety of organelles in axons and dendrites [4], which could lead to HD symptoms: In support of this, HTT silencing decreases organelle motility. In fly models, silencing and knockout of HTT both decrease transport [4] and in mammalian neurons silenced for HTT expression an HTT fragment rescues transport in mammalian neurons, consistent with effects on transport function.

Communication failure, neuronal degeneration, and inflammation

Based on *in situ* and animal model studies, the neuronal consequences of the mutation implicate a cascade of disrupted cellular events, eventually leading to neurodegeneration [4]. Among other mechanisms, protease cleavage of mHTT is known to generate small fragments that interfere with various cellular functions, including transcription [22], transport activity of dynamin 1, autophagocytosis, and calcium signaling.

These results suggest that the N-terminal segment of the protein is crucial c to HTT's various functions and that the mutation interferes with HTT's interactive domains and subcellular processes, e.g. that of vesicular transport.

Consistent with this there is a reduction in vesicular brain derived nerve growth factor in neurons obtained from human embryonic stem cells of heterozygote HD patients. Silencing of the mutant allele, on the other hand, restores transport up to normal values, rescuing the mHTT effect [23], an indication that the intact protein is required to mediate the transport function.

The effects of mHTT are maximal in inhibitory spine projection neurons (SPNs) of the striatum, where there occurs a reduction of GABAergic synapses and subsequent degeneration of the neurons. Early alterations in SPNs in various HD animal models include depolarized membrane potentials, increases in input resistance, and hyperexcitability [24]. In addition, there are differential changes in

glutamatergic, GABAergic inputs, and dopaminergic modulation between the two major classes of SPNs, resulting in an imbalance of the direct and indirect pathways early in disease.

Contributing to these effects is a dysregulation of synaptic genes, based on RNA-sequencing studies in human HD pluripotent stem cell (iPSC)-derived neural cultures. Downregulation of transcripts that participate in the post- synaptic scaffold, neurotransmitter signaling, Ca^{2+} signaling, long-term synaptic plasticity, and neuronal activity- regulated genes have all been observed [25]. Moreover, synapse-related proteins are abundant in the mHTT interactome [26], suggesting that at least some of the synaptic defects are directly caused by the mutant protein, rather than being a secondary consequence of cell deterioration.

The molecular and subcellular dysregulation occurring in pre-manifest HD subjects has been linked to neurophysiological alterations of the striatal circuits. Neuroimaging and electrophysiological studies conducted on pre-HD children, for instance, highlight early striatal developmental alterations as early as 6 years of age. Bold imaging studies, moreover, show changes in neuronal activation even in the absence of striatal atrophy [27], which are observed more than 12 years in advance of overt clinical symptoms. Additionally, calcium imaging of large groups of neurons reveals a statistically significant increase in correlated activity in mice models of HD at early time points before HD symptoms appear. Together, these defects constitute indicators of causal molecular processes involved in HD's subsequent neuropathology and so promising targets for visualization by means of nuclear medicine procedures.

Nuclear Medicine and Receptor Based Mechanisms in HD

To date, PET has provided significant insight into HD dysfunctions, chiefly by targeting neurotransmission mechanisms and neural metabolism [8]. Brain PET has also been used in cases to assess disease induced degeneration via inflammation mechanisms. Among the various categories of brain proteins for which PET imaging has been used to monitor HD are G-protein coupled receptors (GCPR), membrane transporters, ligand gated ion channels (LGIC), tryptophan-rich sensory proteins (TSPO), enzymes, and misfolded proteins [28]. Small molecule- based positron-emitting inhibitors, which bind to the target molecule in the manner of receptor-ligand interaction, for example, are often used as PET tracers for imaging and concentration assessment.

While the use of such tracers provides valuable insight into HD dysfunction, especially disease progression, the imaging information provided is generally not specific to HD pathology nor readily linked to molecular events driven by mHTT [8], as seen with the use of dopaminergic medications for ameliorating HD symptoms. As such the HD induced effects on these synaptic systems appear to be part of a larger suite of secondary influences affecting synaptic proteins and metabolism. Nonetheless, efforts to clarify how these receptor systems are influenced by mHTT are likely to benefit from the development of multiprobe tracers directed to these systems in pre-manifest individuals. This knowledge could provide bridging to molecular events induced by mHTT and/or its downstream partners. A resume of current Pet HD targets follows.

Receptors

Dopamine: Dopaminergic neurons are abundant in the basal ganglia. PET has been used to assess dopaminergic function in both presynaptic and postsynaptic terminals of these neurons. In post synaptic studies, the PET radioligands ^{11}C-raclopride and ^{11}C-SCH23390 help to visualize the distribution of D2, D3 and D1, D2 dopamine receptors, respectively. Use of these tracers has revealed a decline in numbers of striatal postsynaptic D1, D2 and D3 receptors of up to 30% in manifest and prodromal HD subjects [8], which exceeded changes caused by atrophy [29]. The ^{11}C-raclopride and ^{11}C-SCH23390 binding reductions in the striatum were shown to negatively correlate with disease duration in HD individuals. Based on studies with these tracers, degeneration of dopaminergic terminals occurred early during the progression of the disease and was also observed in premanifest cases, suggesting an HD specific effect on dopaminergic synapses related to post synaptic, receptor integrity.

Presynaptic studies have focused on neurotransmitter cellular and synaptic vesicle transporters. The dopamine transporter (DAT) functions in reuptake of dopamine from the synaptic cleft into the presynaptic neuron cytosol and is highly expressed in dopaminergic terminals. Use of ^{11}C-CIT, a specific DAT tracer, in PET imaging has shown that DAT availability in the striatum of HD individuals is reduced by 50% relative to normal controls. The reduction is potentially due to an mHTT induced dysfunction of transport involving the vesicular monoamine transporter–type 2 (VMAT2), which transports monoamines into synaptic vesicles. In line with this, a PET study with the VMAT selective antagonist, ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ), showed reduced striatal binding of ^{11}C-DTBZ in all HD patients compared to normal individuals [7,8].

Glutamate and GABA: Group 1 metabotropic glutamate receptor (mGluR) signaling is altered in presymptomatic HD mice models [30]. A member of the G protein coupled receptor family, mGluR5 is highly expressed in striatal SPNs. Assessment of this receptor in HD subjects can be carried out using ^{11}C-ABP-688, a high affinity, oxime-based structural analogue of the mGluR5 antagonist MPEP. While a role for glutamate receptors in HD has been variously implicated, only a few studies with this or other glutamate-targeting PET tracers have been reported [7].

GABA receptor changes have also been studied in HD, where significant impairment of GABA neurotransmission has been demonstrated [31]. Use of the benzodiazepine-based GABA antagonist and PET tracer, [^{11}C]-flumazenil, shows reduced binding in the caudate with a corresponding loss of projection neurons. However, no differences in binding are seen in the putamen of HD subjects where the loss of neurons is significantly less, suggesting that late occurring cell degeneration may be chiefly responsible.

Opioids: PET imaging with ^{11}C–diprenorphine, a non–selective, partial agonist for the delta, kappa, and mu opioid receptors, has shown reduced binding in the caudate and putamen in manifest HD patients relative to normal controls [7].

Receptor modulators

Adenosine: Adenosine is a modulator of dopaminergic and glutamatergic neurotransmission [32]. It exerts its action through the G protein-coupled receptors, A1, A2A, A2B and A3. A1 receptors are found on dynorphinergic medium spiny neurons (MSNs) which co-express dopamine D1 receptors, while A2A are located on enkephalinergic MSNs that co-express dopamine D2 receptors.

In HD, several studies show correlative A1 receptor binding in relation to years to onset [7] using ^{18}F-CPFPX as a marker of A1 receptors. ^{18}F-CPFPX is a xanthine-based antagonist of the adenosine A1 receptor with a binding selectivity over A2A of roughly 1200-fold in HD. In early pre-manifest patients, tracer binding is elevated by roughly 35%, while late pre-manifest patients display comparable binding to controls. In manifest patients tracer binding continues its downward movement as seen by the significant binding reduction at this stage of about 25%.

Cannabinoid: Cannabinoid receptor type 1 (CB1) is expressed abundantly in striatal MSNs and belongs to the G protein-coupled family. CB1 is involved in modulating inhibitory neurotransmission [33]. Assessments of CB1 show a striking loss of CB1 receptors in post mortem brains of HD patients, which is greatest in the caudate nucleus, putamen and globus pallidus externus, even in the very early stage of the disease. The radiolabeled tracer, ^{18}F- MK9470, specifically binds to CB1. Use of this radiotracer in symptomatic HD patients reveals a significant reduction in CB1 receptors relative to controls, although the reduction is not correlated with HD motor scores or disease [7].

Phosphodiesterase 10A: Phosphodiesterase 10A (PDE10A), catalyzes the deactivation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Phosphodiesterase 10 (PD10) is highly expressed by the medium spiny neurons in the striatum and is believed to play a role in modulating dopaminergic and glutamatergic neurotransmission. Binding of ^{18}F-MNI-659, a

specific radioligand for PD10, is reduced in the basal ganglia of HD individuals relative to controls and in animal models PD10 expression is thought to be suppressed by mutant HTT. Reduced binding in the striatum in pre-manifest individuals has been demonstrated using another highly selective radioligand, ^{11}C-IMA–107, suggesting that PD10 changes occur several years before the manifestation of HD symptoms [34].

Metabolism and inflammation

^{18}F-fludeoxyglucose (FDG) has been employed in PET imaging to monitor glucose metabolism [7,8]. In subjects with HD, PET studies have revealed an annual decrease in glucose metabolism across premanifest and HD individuals compared to normal controls, suggesting that hypometabolism in HD may precede clinical manifestation of the disease.

Besides hypometabolism, there are other secondary effects of HD, including inflammation and neurodegeneration. Such effects result in the activation of microglia that proliferate and change their morphology, gene expression and function [8,35]. Currently, radiolabeled tracers for microglial activation target the translocator protein (TSPO), an 18 kDa protein chiefly found in the outer mitochondrial membrane. TSPO is highly expressed in activated microglia compared to the normal microglial state, providing a robust determination of microglial activation.

Nuclear Medicine Imaging Advances and HD

Progress in characterizing the molecular features of HTT and its cellular roles in brain neurons has helped to clarify new areas where advances in nuclear medicine technology can assist research and diagnosis of the disease, including technology advances likely to enhance the dynamics and quantitation of radiopharmaceutical and radioligand probes. Included are such improvements as multiprobe and multimodal information processing, signal acquisition and system sensitivity and stability, dynamical and quantitative assessment, and computational proficiency (Table 1). Several are being employed in the medical management of HD.

Table 1

Prospective Advances in Nuclear Medicine Instrumentation and Tracer Methods for HD

	Designation	Technical Advance	Medical Objective
Instrumentation and Computational Advances	Multimodal		
	PET/MRI	Multimodal	Improved PET spatial resolution
	PET/fMRI	Multimodal	Network influences
	PET/MRS	Multimodal	Radiogenomics/Preclude biopsy
	PET/OI	Multiprobe radioimaging	Surgical guidance
	Small gamma cameras	Reduced camera size	Surgical guidance
	Image reconstruction		
	Reconstruction free	Rapid TOF	Reduced computation
	Attenuation correction	MRI based correction	Image recovery
	Reconstruction algorithms	AI pattern recognition	Improved registration
	Quantitation		
	AIF/ROI quantitation	Non-invasive	Kinetic analyses
	Principal Components	Voxel factoring	Patient Stratification
Radio-probes and Targeting Advances	Blood Brain Barrier		
	Nanoplatforms	Design based vectors	Transport through BBB
	Viral vectors (rAVV)	Natural brain selective vectors	Transport through BBB
	Hyperpolarization contrast	Enhanced molecular signal	Diagnostic/Species localization
	Radioimmunoconjugates.	Intrabodies/IgG fragments	Suppressed Protein-protein interactions
	Reporter genes	Select gene expression	Protein distribution/function
	Genetic probes	Expression modulation	Gene product modulation

Abbreviations: PET, positron emission tomography; MRI, magnetic resonance imaging; fMRI, functional magnetic resonance imaging; MRS, magnetic resonance spectroscopy; OI, optical imaging; TOF, time of flight; AI, artificial intelligence; AIF, arterial input function; ROI, region of interest; rAVV, recombinant adeno-associated viral vector

Multiprobe and multimodal technologies

PET/MRI

Combining (PET) and (MRI) into a single device that can acquire both datasets simultaneously has been an objective for several decades. Simultaneous acquisition provides for the temporal correlation of data sets, which, given the dynamic nature of PET measurements, can undergo substantial change during the interval between dataset readings and must be accurately adjusted for in co-registration procedures. Accordingly, in patients requiring both PET and MRI analyses, simultaneous acquisition can be expected to enhance spatial registration between the two image modalities as well as increase patient comfort.

The techniques of PET and MRI provide complementary types of information about the brain. On the one hand, PET can yield insight into the physiological and metabolic features of brain tissue by tracking the distribution of molecules *in vivo* using radionuclide positron emission. Because of the nature of the emission, however, spatial resolution is limited, restricting the ability of PET to localize events that radio-tracers are monitoring. MRI, on the other hand, generates precise anatomical and structural imagery that has superior contrast in soft tissue. The evolution in modern MRI techniques has additionally exploited other sources of endogenous contrast to monitor function (functional MRI), physiology (diffusion tensor MRI), and composition (magnetic resonance spectroscopy) [11].

To avail the advantages of simultaneous imaging, however, several technical challenges needed to be overcome that led to significant changes in instrumental design. For PET imaging a chief obstacle involved magnetic field interference on the performance of photomultiplier tubes. In early modifications this obstacle was addressed by integrating PET detectors in specialized MRI scanners such as split-magnet or field-cycled units [11]. Later and more successful modifications were achieved with the development of MRI-compatible photon detectors that could be placed inside the magnet's bore. Avalanche photodiodes, for example, were capable of functioning in the presence of even ultra-high magnetic fields, allowing their use in brain imaging [36]. Solid-state photomultipliers (SSPM), silicon photomultipliers (SiPMs) or multi-photon pixel counters (MPPC)) are now the photon detectors of choice. Most PET systems use lutetium oxyorthosilicate (LSO) or lutetium yttrium oxyorthosilicate (LYSO) as scintillator materials [6]. For MRI imaging, a major hurdle to combined modalities involved the maintenance of magnetic field homogeneity with placement of PET components in the MRI scanner bore. To overcome this hurdle only non-magnetic versions of working components are now used. Additionally, electromagnetic interference in the radiofrequency range is minimized and the shielding designed to avoid currents due to changes in the magnetic gradient during scanning.

MRI as a structural framework for PET in combined systems

Improved spatiotemporal accuracy of PET estimates has been achieved by using MRI data to provide a structural framework on which the simultaneously recorded PET signal is distributed. Combining these with adjunct computational analyses such as time of flight localization and principal components analysis has been shown to further enhance accuracy.

Time of flight localization: Time of flight information (TOF) narrows the spatial location of positron emission, thereby improving the spatial resolution of emitted signals. In principle, time of flight computations rest on the detection of the physical annihilation of the positron and the generation of two 511 kEV photons that are separated by 180 degrees. The computational steps are straightforward and use the following equation:

$$D1 - D2 = (t1 - t2) \times c$$

Where c is the speed of light and $t1$ and $t2$ are the recorded detection times. Uncertainty in the time measurements is incorporated in computations by use of the TOF probability distribution.

Time of flight technology has undergone a significant improvement in localization capability due to the evolution in silicon photomultiplier tubes and the use of scintillators with improved performance, such as those containing lutetium oxyorthosilicate (LSO)

or lutetium yttrium oxyorthosilicate (LYSO). With these materials, the current coincidence time capability of resolution is listed at 200 pico-seconds, with experimental systems achieving nearly double that at 100 pico-seconds. Given ongoing developments, time of flight determinations may eventually prove sufficient for clinically precise localization, obviating the need for reconstruction algorithms [6].

Quantitative evaluation of PET data using MRI

Attenuation correction: Attenuation correction in procedures such as PET/CT scanning can be directly determined based on photon attenuation in the tissue medium and its conversion via 512 keV linear attenuation coefficients. MR based attenuation correction in combined PET/MRI, however, is challenging due to MRI datasets that are based on proton density and relaxation rates rather than on electron density. The latter must therefore be inferred to provide for PET attenuation. Accordingly, several methods have been developed to correct for tissue attenuation in PET/MRI.

One class of methods employs PET emission data to estimate attenuation data via iterative joint estimation, based on maximum likelihood (ML). Other procedures derive attenuation data from MRI based information. These may use precompiled atlas pairing, which relates MRI and PET images via an algorithm, or direct imaging using Dixon, ultra-short echo (UTE) or zero echo time (ZTE) methods that avoid use of complex imaging registration and processing procedures. In the direct method individual patient MR images are segmented into several tissue classes with individual tissue classes assigned a constant or continuous attenuation factor value. In the direct 2 point Dixon method [37], for example, two different echo times are used based on the different precession rates of fat and water molecules. This method, however, does not compensate for lung and bone readings, which thus distorts the attenuation correction. An alternative and frequently employed approach is the use of an ultra-short time echo (UTE) sequence, with acquisition times approximately 100 fold shorter than echo times typically used in *T*1-weighted MR images [38]. Using these techniques, for example, a recent region of interest analysis of inflammation in HD with the tracer ^{11}C-PBR28, showed statistically significant differences between manifest patients and controls in the pallidum and putamen regions of the brain [12].

Principle components: Objective methods for PET typically treat voxels as if they represented independent or uniformly correlated measures throughout the brain, an unwarranted presumption given the known structural variation within the brain. Principal components analysis assists in accounting for this variation, reducing the original dimensionality of imaging data to a suite of low dimensional contributing features. Individual component axes can then be identified as to the magnitude of feature contribution to signal variation within a PET image.

In practice, data are typically transformed into a (n x v) data matrix where n is the number of image observations and v is the number of voxels. Principal components and their variance are obtained from the eigenvectors and eigenvalues, respectively, which are determined from the correlation matrix of the data matrix [39]. One of the important features of PCA is to provide quantitative coordinates for the observations on uncorrelated axes. The coordinates are then quantitatively related to attributes of the individuals. In a principal components study of Huntington's disease subjects, for example, atrophy of the caudate was found to be a significant contributor to the total correlation as a function of the stage of progression [12]. The identification of this component - along a first PCA axis - was sufficient to classify HD subjects according to their disease status.

Image-based radiotracer AIF estimation and radiotracer delivery quantitation: Accurate PET quantification requires an input function to compartment models used for estimating parameters of interest for normal and pathologic changes in tissue function or metabolism. For example, a plasma time-activity curve of tracer delivery to the tissue is typically derived from a radiotracer arterial input function (AIF) [6,40]. Because the determination of the AIF involves radial artery catheterization, however, its use is limited in routine PET studies. Accordingly, noninvasive image-based techniques have been proposed.

One such method is a derivation of the AIF determined from blood vessel, regions of interest obtained after administering a tracer. Correctly defining the region of interest over a vessel together with confounding effects can be challenging using PET images only, however. This drawback has been addressed and circumvented in a combined scanner, where coregistered and simultaneous MRI anatomic images can be used to accurately measure the position and size of the vessels of interest. With coadministration of both MRI contrast and PET tracers, MRI can also provide information about the dynamics of bolus delivery to the tissue of interest and assess any local changes in blood flow, thereby reducing the effects of bolus delay and dispersion in the AIF estimate [6].

Dynamic acquisition of uptake and elimination of tracer over time additionally allows for kinetic analysis of data sets, which may be clinically relevant. With combined PET/MRI systems, kinetic models can be developed that use both dynamic PET and dynamic MRI data for parameter estimation. These systems also provide for non-invasive estimation of the input function, which is needed for kinetic modelling.

MRI based PET motion correction

Simultaneous PET/MRI enables spatial and temporal correlation not accessible by sequential or parallel methods. This is of significance in neurologic and psychiatric diagnosis, where MRI is typically a firstline modality and many brain PET tracers are now available. Cross correlation can correct, for example, spatial and temporal changes that may be due to physiological or subject motion, anatomical variability, or dynamical event transitions [11]. On the other hand, most of these methods require a relatively unobstructed view of the optical sensors from outside the scanner-which is difficult to achieve in an integrated PET/MRI scanner due to the presence of the radiofrequency coils.

For more conventional acquisition methods (e.g. high-resolution anatomic imaging), motion estimates with very high temporal resolution (e.g. every 20 ms) can be obtained using embedded navigator pulses. Their use to correct PET data in very short frames is especially important for performing motion correction in the early phases of a dynamic PET study, when frames as short as 1 s are often used to sample the radiotracer AIF.

PET/fMRI

Functional magnetic resonance imaging (fMRI) evolved to capture dynamic brain events with high spatial precision. Recent combined sensing using fMRI with PET have begun to link molecular and subcellular information to functional, systems level dynamics, with the prospect of offering novel perspectives into the brain's function and dysfunction [41]. In Parkinson's disease, for example, simultaneous PET and MRI signaling can relate complex dopamine mechanistic responses such as the rates of release or transport (PET) with neuronal activation (fMRI).

Given that PET radiotracers have already provided valuable information about dopaminergic, neuroinflammatory, phosphodiesterase, metabolic, and other functional targets in HD [8], PET/fMRI can be expected to provide key insights into molecular events leading to circuit and network dysfunction.

PET with task based and resting state fMRI

Increasingly, non-invasive, task-based regimes are employed to monitor the functional consequences of perturbing the brain's network organization, including repetitive transcranial magnetic or direct-current stimulation. In conjunction with PET imaging, these regimes could be used to activate or inhibit select areas of the cortex [41].

From its origins in the 1990's, resting state fMRI has evolved into a powerful and spatially accurate tool for assessing the functional organization of the brain. The early detection of resting state networks by Biswal., *et al.* [42] used a standard 1.5 T clinical scanner

equipped with a three-axis head gradient coil that obtained images every 250 ms. Under these relatively moderate scanning conditions, they showed the presence of a high degree of temporal correlation in brain activity of the sensorimotor cortex and several other regions associated with motor function in resting patients. Current procedures typically employ 3.0 T for better spatial resolution and use parallel imaging for fast data access. In special cases, high strength magnetic fields (7 T) and big data acquisition procedures are used [43].

Use of resting state fMRI has revealed that brain connectivity of the left middle frontal and pre-central gyrus, and right post central gyrus with the medial visual network is reduced in premanifest and manifest HD as compared to controls ($0.05 > p > 0.0001$) [44]. In manifest HD the connectivity of numerous widespread brain regions with the default mode network and the executive control network are reduced ($0.05 > p > 0.0001$). These differences are present even when the potential influence of atrophy is taken into account, suggesting that pre-manifest synaptic or corresponding subcellular events have impaired circuit function.

PET/MRS

Magnetic resonance spectroscopy (MRS) is a non-invasive technique used for *in vivo* measurement of levels of unique molecular species, such as total choline (tCho), a marker of neoplastic proliferation [45,46]. In MRS the magnetic field experienced by a particular nucleus is affected by the motions of its nearby electrons, in contrast to MRI where the magnetic moments of nuclei become oriented relative to the direction of the applied field. Hence, differently sited nuclei experience slightly different applied fields and resonate at slightly different frequencies, generating unique fingerprints for different compounds. Single-voxel MRS methods have been used to compare levels of such compounds in tumors and in adjacent or contralateral normal brain tissue in neurodegeneration.

PET/MRS combined modalities offer the prospect of simultaneous molecular assessment by two independent signal sources. The use of MRS imaging (MRSI), however, has generally been hampered by its low sensitivity *in vivo*.

Recent developments using hyperpolarized MRS have addressed this obstacle, enabling wider use of combined PET/MRS. With hyperpolarization signal strength can be increased by many orders of magnitude. PET/MRS studies with a combined hyperpolarized, ^{13}C-labeled pyruvate substrate have confirmed its capability for imaging tumor metabolism [47], suggesting the feasibility of employing ^{13}C-MRSI in the clinic for HD.

Multiple hyperpolarization technologies have been developed for biomedical applications [48], including dissolution Dynamic Nuclear Polarization (d-DNP), Parahydrogen Induced Polarization, Signal Amplification by Reversible Exchange (SABRE), and Spin Exchange Optical Pumping. The chief objective of the hyperpolarization process is to yield sufficient hyperpolarized contrast agent (HCA) having an adequate lifetime (i.e. long T1) for *in vivo* distribution and metabolism. Because protons typically have low T1 values - on the order of only a few seconds - most HCA include a low-γheteronucleus (129Xe, 13C, 15N, 3He, etc.) for hyperpolarization storage and detection.

In cases where sensitivity is sufficient, various nuclear signatures can be used in PET/MRS for detection of a wide range of metabolite markers of functional integrity. Use of 31P-MRS, for example, enables detection of phosphorylated metabolites, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), and phosphocreatine.

Given numerous animal models currently available for the study of HD, use of PET/MRS could potentially be used to identify affected molecular pathways leading to network dysfunction. For example, 1H-MRS studies have reported a decrease of the total N-acetyl-aspartate (tNAA) concentration in the striatum and cortex of R6/2 mice, an HD mouse model.

PET/MRS and radiogenomics

Radiogenomics is a novel approach to combined PET/MRS nuclear isotopic and nuclear moment modalities that interrogates large scale relationships, termed association maps, among imaging phenotypes and molecular data sets, such as those from specific gene and microRNA expression signatures. Association maps can identify high dimensional, correlation relationships, which can entail either simple discrete measurements or complex combinatorial data, such as those between high dimensional image features and large scale molecular or genomic elements. Such relationships constitute potential biomarkers of disease, and the information can be used for guiding treatments in the absence of new pathologic specimens [13].

PET/OI

PET and optical imaging (OI) dual modalities have developed chiefly in the context of surgical guidance, which are prospectively suited to the introduction of probe vehicles in HD. Optical imaging types now available comprise a range of optical modalities [6], including fluorescent protein molecules, Cherenkov luminescence imaging, near- infrared light, and quantum dots composed from Cd/Te or Cd/Se materials. In the context of surgical guidance, the radioactive signal enables identification and localization of a lesion by means of its radioactive signature, with the optical feedback allowing direct lesion visualization and delineation in exposed tissue.

While the use of combined PET/OI has seen little application to date in HD, therapeutic procedures involving genetic therapies are under development [49] that are likely to require such diagnostic tools for localized targeting and for assessing the effectiveness of therapy. Therapeutic strategies for decreasing mHTT levels have notably been effective in mouse models, which could be interrogated in clinical studies for humans.

Computational and technological advances

Enhanced signal acquisition

Important improvements in sensitivity and resolution of PET technology have included more rapidly responding and brighter scintillators that are based on materials like lutetium oxyorthosilicate (LSO), gadolinium oxyorthosilicate, and lutetium yttrium oxyorthosilicate [50]. Coupled with the development of solid-state read-out detectors, new scintillators now enable 3-dimensional data acquisition and use detector material composed from cadmium zinc telluride (CZT).

Small-field planar gamma cameras

Over recent decades, the use of small hand-held, planar gamma cameras designed for surgery has increased [48]. Given the need for genetic therapies in HD treatment, the smaller cameras are likely to see more frequent use in these procedures, which involves the targeted delivery of probe material [49]. Several groups have developed CZT- based intrasurgical systems and a range of cameras has become commercially available. In contrast to the use of probes, gamma cameras enable more precise viewing of a region of interest and can be combined with optical cameras to facilitate interpretation.

Computational and processing software and AI

Reconstruction software

Developments in image reconstruction continue to play an integral role in achieving optimal image quality for both SPECT and PET. In most cases iterative reconstruction [51] is used, since the contribution from attenuation, motion, and detector resolution are sufficiently large as to appreciably diminish image quality. In other cases reconstruction can be performed more rapidly by filtered backprojection techniques. The improvement in speed with this procedure, however, necessitates the use of smaller data sets to achieve improved computational speed, potentially lessening image quality.

Since all such approaches require significant computational tasking, albeit at different scales, recent proposals have advanced the concept of reconstruction free PET imaging [52] to reduce computation. Reconstruction free imaging is based on the notion that with sufficient improvement in time of flight information, the need for reconstruction will be obviated, after application of a relatively few analytic corrections to the cross-sectional PET images. A recent demonstration of an imaging system based on the detection of Cherenkov photons in two collimated detectors, in fact, had a coincidence TOF resolution of 32 ps3, which was argued to be sufficient for reconstruction free imaging [52].

Radioligand Advances in HD Diagnosis and Therapy

While most radionuclides for HD target receptor and/or receptor linked mechanisms, the increasing knowledge of Huntington's complex molecular pathogenesis has revealed new ways in which its etiopathology can be more directly assessed and treated [9,49]. A number of these methods can target the protein itself or its partners, with such diagnostic objectives as localization, expression and modification, turnover and clearance, molecular associations, intracellular functioning, and extracellular distributions (Table 1).

Transport across the blood brain barrier

A fundamental limit on the use of radionuclides in the brain is the blood brain barrier. All radioligands for Huntington's Disease require, therefore, that the design of radioligands incorporate features enabling the ligand to overcome this barrier. For many radioligands this can be achieved by the construction of compounds sufficiently low in molecular weight, generally not exceeding 500 daltons. For example, antisense oligonucleotides for huntingtin, which have been used in research and clinical trials, are typically designed not to exceed this size range [6,49].

In other cases, entry to the brain has required the use of novel methods that do not rely on size. Large compounds, e.g. immunoglobulins, may require attachment to physical vehicles capable of being taken up into brain tissue.

Developments in nanotechnology offer such a platform for incorporating radionuclide ligands, which can be coupled to efficient nano-carrier systems [14]. By manipulating some of the properties of these nanoparticles (NPs), suitable agents can be attached or loaded. A drawback to the use of nanoparticles, however, has been an inability to target tissue destination, resulting in their dissemination to multiple tissues rather than to the brain alone [53]. One scheme to overcome this drawback harnesses the lower endocytic rate of brain endothelial cells to promote selective retention of free, unconjugated, protein-binding ligands on brain endothelial cells. Nanoparticles capable of binding to the displayed ligands are thereby targeted specifically to the brain microvasculature rather than locating in peripheral organs.

Besides nanoparticles, viral vectors are also used to deliver radionuclides. Viral vectors for neurological tissue now include a broad spectrum of vectors derived from multiple viral classes, including retrovirus, lentivirus, adenovirus, herpes simplex virus type 1 (HSV-1) and AAV vectors, with recombinant adeno-associated virus (rAAV) vectors generally finding more frequent use [54]. Recombinant AAV vectors feature a number of advantages. They are nonreplicable, non-pathogenic, and do not integrate into the host genome [11]. Pre-clinical *in vivo* AAV gene therapy studies, for example, have been carried out for HD involving both shRNA and miRNA approaches [54].

Radioimmuno-diagnostics and radioimmuno-therapeutics in HD

Designing novel immunotherapeutics for HD can be expected to be much more effective given the increasing knowledge of the HTT protein and its functional partners. A wide range of sites on the protein have been used for immunogenicity. These include such functionally significant domains as the N-terminal region and the poly-proline and polyQ segments, all within exon 1 and all of which have been identified as influencing protein toxicity, as well as various protein modification sites within the protein [4]. With current techniques, antibody binding to protein sites can be obtained both within and outside cell compartments [9].

Radioimmuno-diagnostic or radioimmuno-therapeutic tracers are constructed from monoclonal antibodies and their derivatives, which affords narrowly focused targeting of molecular and cellular species. When combined with radionuclides, it is possible to obtain critical information on antigen quantitation, heterogeneity, and kinetics in real time. Numerous radionuclides are currently available, including actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth- 213 (^{213}Bi), indium-111 (^{111}In), iodine-123 (^{123}I), iodine-124 (^{124}I), iodine-131 (^{131}I), lead-212 (^{212}Pb), lutetium-177 (^{177}Lu), technetium-99m (^{99m}Tc), copper-64 (^{64}Cu), gallium-68 (^{68}Ga), yttrium-86 (^{86}Y), yttrium-90 (^{90}Y), and zirconium-89 (^{89}Zr). The choice of radionuclide may be based on several properties that depend on need and objective, which can include emitter type, energetics, and half-life, as well as tissue characteristics. Efficient radiolabeling is often carried out by binding the radionuclide to a bifunctional chelator, which possesses a binding site for the radionuclide and a linker that can attach a nucleophilic group to a carrier antibody [55].

While radioimmunoconjugates have yet to see significant use in HD, there is a rapidly increasing number of protocols that employ antibodies to HTT for research and therapies used in managing the disease that could be easily coupled with selected radioligands for diagnostic and therapeutic protocols. For example, it is known that the mutant protein is abundant in free forms and is distributed among several compartments, including the cerebrospinal fluid, plasma, and extracellular matrix. This distribution has appealed to the use of antibodies to the protein for reducing toxicity [9], an approach that could be directly assayed by means of the radioimmunoconjugate.

Several classes of antibody therapies have been developed, which are differentiated based on their respective targeting, antibody protocol, and location.

Therapies targeting intracellular mHTT

Intrabodies targeting intracellular mHTT were developed in the late 1990s. They are typically generated by recombinant gene techniques to yield the smaller antigen-binding fragment and introduced to cells via adeno- associated viruses (AAV), which manufactures the fragment within the cells. Inside the cells the manufactured fragments interfere with or otherwise neutralize the protein's functional domains [56]. The first intrabody developed for HD, for example, was specific for the 17 amino acid, N terminal exon 1 domain of the soluble form of HTT/mHTT and was shown to inhibit aggregate formation in cell cultures and increase lifespan in a drosophila HD model.

Targeting extracellular mHtt

Considerable evidence reveals the involvement of mHTT in extracellular events that influence the severity, symptomatology, and propagation of HD across cell systems. Among the various mechanisms proposed for influencing these features are effects on brain related immunogenicity, a seeding pathology that amplifies mHTT mobilization, and prion like activity [9]. The extracellular antibody targeting of these aspects has the advantage of requiring simpler protocols than those needed for cell entry, such as actively exposing the immune system to the mutant form of HTT, which makes it a desirable protocol objective.

Targeting free/extracellular mHTT can be carried out by passive or active immunization. In active immunization the immune system is exposed to an exogenous antigen to elicit an adaptive immune response. This process has the advantage of generating an acquired immune response that is relatively long lasting. In passive immunization exogenous antibodies are introduced to suppress antigenicity. While passive immunization generates a relatively rapid response, the introduction of exogenous antibodies lasts for a much shorter interval than active immunization.

Complement directed therapeutics with C1q neutralizing antibody

Synaptic removal is known to be mediated in part by mechanisms of the classic complement pathway. In neurodegenerative disorders like HD, complement mechanisms become dysregulated, resulting in excess synaptic loss and dysfunctional circuitry. Several therapies

using antibodies have been developed that could employ radioligands for evaluating the pharmacokinetics and pharmacodynamic effects on synaptic removal and complement processes, e.g. the Annexon anti-C1q monoclonal antibody ANX-005 [9].

Clearance mechanisms in HD: Radiolabeling of ubiquitin interactions

By binding to the p62-interaction domain in HTT, HTT promotes the recognition of ubiquitinylated proteins at the p62 site [57], facilitating cargo loading into phagosomes. A defect in loading of the autophagosomes in HD leads to a reduced capacity to degrade aggregated proteins [4]. In animal models carrying the HD mutation the lowered capacity has been shown to generate protein inclusions that are coincident with the progression of neurological symptoms. The inclusion bodies (IBs) are typically decorated with ubiquitin, proteasomes, and chaperones, revealing that protein clearance mechanisms for homeostasis are disrupted.

Radiolabeling of ubiquitin offers significant potential for monitoring clearance mechanisms that have been altered by mHTT. It is known, for example, that mHTT inclusion bodies recruit catalytically active enzymes involved in ubiquitination and (de)-ubiquitination processes. Procedures for ubiquitin labelling have been developed for study of breast tumor cancers in animal models [58] and could find application in HD. In one procedure ubiquitin was labeled with 64Cu via a linker sequence. The ubiquitin was first fused with a C-terminal GGCGG sequence, and the resulting recombinant ubiquitin derivative UbCG4 was then functionalized prior to 64Cu-radiolabeling.

Assessing genetic influences of mHTT: DNA and RNA expression probes

Transcriptional effects mediated by mutant huntingtin include, among others, the selective impairment of DNA repair, and apoptotic and metabolic pathways involving the PGC, HSP70, and CREB promoters. Proposed mechanisms for the observed effects involve transcriptional repression either of the promoters or of the promoter regulated genes [4]. For example, mutant huntingtin is known to repress the CRE-mediated transcription of PGC-1a by interfering with the CREB/TAF4 transcriptional pathway in striatal neurons.

Therapies involving lowered mHTT expression target the gene and/or its expressed transcriptional product.

For HD, nucleic acid based approaches have used gapmer, anti-sense oligonucleotides (ASO) and anti-sense oligonucleotides [49,59]. These target exon 36 in the gene to lower huntingtin levels. The former are a string of nucleotides with a central unmodified region flanked by modified nucleotides. Both approaches result in RNase H degradation of mutant and wild-type HTT transcripts.

mHTT procedures are potentially assayable by means of radiolabeling. In the case of nucleic acid-based medicines, radiolabeling requires that several factors be considered to optimize signal level and selectivity.

Notably, each of the three isotopes primarily used for radiolabelling of oligonucleotides, 3H, 14C, 35S, present difficulties for imaging [55], which must be considered. For instance, the relatively long terminal elimination half- life of oligonucleotides, in some cases 30 - 60 days or even longer, poses a significant disadvantage affecting the consideration of metabolic stability. Specifically, 14C needs to be incorporated in the C2 pyrimidine position to achieve sufficient activity for detection and placement of 3H in the C-8 position of purines poses a risk of tritium- hydrogen back-exchange with the formation of tritiated water, particularly under alkaline conditions.

Reporter gene radiolabeling: Assaying mHTT and protein partner interactions

The multiplicity of processes known to engage HTT and its large size have suggested that the protein functions as a hub tethering multiple partner complexes, which are dynamically modulated by signal transduction mechanisms to activate different processes in space and time. For example, HTT is known to modulate the assembly of dynein/dynactin complexes, with dynein/dynactin scaffolding subject to regulation by HTT phosphorylation [4].

A key question has been that of which sets of protein interactions mediate these cellular processes and how these become dysfunctional in HD. The large number of differential interactions distinguishing mHTT from HTT [60] suggests that monitoring these interactions *in vivo* can be expected to provide critical information about the corresponding impairments in these processes in HD. Increasingly, reporter gene imaging has become an important tool for introducing radiolabeled imaging agents into cells to assay the activity of specific cell processes. When introduced into target cells, reporter genes produce a protein receptor or enzyme that binds, transports, or traps a subsequently injected imaging radioprobe, which becomes the contrast agent [61]. Currently, recombinant adeno- associated viral (AAV) vectors are the most frequently used delivery method for effective cell transduction and stable expression of a modified gene.

Several gamma-emitting radionuclides are available for radiolabeling injected agents, from small molecules and peptides to antibodies, nanoparticles and cells. In the clinic, the most widely used radionuclide is ^{99m}Tc which has a moderately short half-life (6 hours), which is long enough for convenient synthesis of radiotracers while not imposing prolonged radiation exposure to the subject, favorable emission properties, and convenient production methods. Because of its metallic character, ^{99m}Tc radiotracers employ coordination complexes to bind the radionuclide with a chelating agent.

Despite significant limitations initially with the biodistribution and specificity of reporter gene products, there is a growing repertoire of available reporter genes that could be used for tracking the interactions of mHTT with its protein partners. Following the advent of standardized genetic editing techniques, researchers have isolated a large collection of reporter and modifier proteins (RPs and MPs, respectively) from a variety of species that have since been instrumental in characterizing a wide range of biological processes [10]. For example, RPs are frequently used to tag endogenous proteins or to track the behavior of individual cells *in vivo*.

A particularly promising avenue employs radiolabeled antibody fragments as reporter probes [10]. Antibody fragments retain the ability to target specific protein domains that may be critical in mediating protein-protein interactions and are more accessible to restricted sites due to their smaller size than whole antibodies. In broad terms, the mode of action of intrabodies is to suppress protein activity by directly binding to its functional domain and thereby interfere with the ability of a pathological protein to interact with binding partners or, in cases, to redirect the pathogenic protein to clearance processes [56]. The first intrabody for HD was generated from a human spleen, single- chain variable fragment (scFv) phage library [62], with a number since developed for following HTT/mHTT protein domains, including polyQ, polyProline, and N-terminal exon 1 domains, as well as for mHTT aggregates.

Conclusion and Future Directions

Given the monogenetic nature of HD, early dysfunctions are of major importance, because patients will go on from these to develop the characteristic and devastating clinical manifestations of HD. Study of early dysfunctions is benefiting from a deepening understanding of huntingtin's molecular features and functions and its dynamic interactions with other proteins in what are known to be large scale molecular assemblies. Such understanding may eventually lead, for example, to the identification of markers of disease progression that could be used to validate prodromal neuroprotective and manifest restorative therapies. Accordingly, a major argument for the use of nuclear medicine in Huntington's Disease is that *in vivo* knowledge of disease related changes in such molecular processes is likely to give access to pathological steps occurring early during disease progression that may be beneficial for diagnostic and therapeutic purposes.

Advances in PET technology and radiolabeling are prospectively offering unparalleled access both to the dynamics of the huntingtin protein and downstream events affected by the HTT mutation and to clarification of how these early events lead to circuit and network dysfunction. Technological advances are capable of multimodal data streams and high dimensional analysis that are assisting in achieving the holy grail of personalized medicine.

Improved access and tracking with nanoparticles and viral vectors, reporter genes, and radioimmunoconjugates will enable precision guided therapies and cell specific diagnoses. Importantly, the combination of nuclear medicine techniques at molecular scales with functional imaging at network scales will help to inform the etiopathological sequence from mHTT to brain dysfunction.

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Volume 17 Issue 7 July 2025