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Components of the endocannabinoid and dopamine systems are dysregulated in Huntington's disease: analysis of publicly available microarray datasets

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Abstract

The endocannabinoid system (ECS) and the dopaminergic system (DAS) are two major regulators of basal ganglia function. During Huntington's disease (HD) pathogenesis, the expression of genes in both the ECS and DAS is dysregulated. The purpose of this study was to determine the changes that were consistently observed in the ECS and DAS during HD progression in the central nervous system (CNS) and in the periphery in different models of HD and human HD tissue. To do this, we conducted a meta-analysis of differential gene expression in the ECS and DAS using publicly available microarray data. The consolidated data were summarized as observed changes in gene expression (OCGE) using a weighted sum for each gene. In addition, consolidated data were compared to previously published studies that were not available in the gene expression omnibus (GEO) database. The resulting data confirm gene expression changes observed using different approaches and provide novel insights into the consistency between changes observed in human tissue and various models, as well as disease stage- and tissue-specific transcriptional dysregulation in HD. The major implication of the systems-wide data presented here is that therapeutic strategies targeting the ECS or DAS must consider the dynamic changes in gene expression over time and in different body areas, which occur during HD progression and the interconnectedness of the two systems.

Abbreviations

2-AG, 2-arachidonyl glycerol; ABDH, abhydrolase domain-containing protein; AEA, anandamide; CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; CNS, central nervous system; COMT, catechol-o-methyl transferase; COX-2, cyclo-oxygenase-2; CPu, caudate and putamen; DAGL, diacyl glycerol lipase; DARPP-32, dopamine and cyclic AMP-regulated phosphoprotein 32 kDa; DAS, dopaminergic system; DAT, dopamine transporter; DBH, dopamine β-hydroxylase; DDC, DOPA decarboxylase; D_RD_X, dopamine receptor type x; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; GDE1, glycerophosphodiester phosphodiesterase 1; GEO, gene expression omnibus; HD, Huntington's disease; MAO, monoamine oxidase; MGLL, monoacyl glycerol lipase; mHtt, mutant huntingtin protein; NAAA, *N*-acylethanolamine acid amidase; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D; OCGE, observed change in gene expression; PAH, phenylalanine hydroxylase; PPARα, peroxisome proliferator-activated receptor α; PTPN22, protein tyrosine phosphatase nonreceptor type 22; THC, Δ^9-tetrahydrocannabinol; TH, tyrosine hydroxylase; TRPV1, transient receptor potential vanilloid 1; VMAT, vesicular monoamine transporter.

Introduction

Huntington's disease (HD) is caused by the expansion of a polymorphic CAG trinucleotide repeat in the first exon of the gene encoding huntingtin (Huntington's Disease Collaborative Research Group (HDCRG) 1993). Expression of mutant huntingtin protein (mHtt) leads to a characteristic triad of highly disabling motor, psychiatric, and cognitive symptoms that progress over several decades leading to death (Newcombe 1981; Adams et al. 1988; Roos et al. 1993; Foroud et al. 1999) Pharmacotherapy for HD patients is currently very limited and disease progression is relentless (reviewed in Ross et al. 2014). At the cellular level, expression of mHtt containing an expanded CAG repeat-encoded polyglutamine region in the amino terminus of the protein leads to a variety of changes in cellular function including transcriptional dysregulation (reviewed in Zuccato et al. 2010).

Because specific subsets of caudate and putamen neurons (CPu) are known to be particularly susceptible to cell death in HD, there has been an intense effort to define changes in gene expression in the basal ganglia and particularly the medium spiny projection neurons in the CPu (which is functionally equivalent to the striatum in rodents; Graveland et al. 1985; Vonsattel et al. 1985; Halliday et al. 1998). Further, the chorea, akinesia and dystonia that occur in HD suggest profound basal ganglia dysfunction. Early studies that looked at levels of individual mRNAs and proteins demonstrated that the levels of a subset of mRNA and proteins were altered when mHtt was expressed (Augood et al. 1997; Cha et al. 1998; Denovan-Wright and Robertson 2000; Glass et al. 2000). In addition, multiple microarray studies have reported profound and early transcriptional dysregulation in the basal ganglia of HD patients (Luthi-Carter et al. 2002; Desplats et al. 2006; Hodges et al. 2006). One hypothesis is that the amino terminus of mHtt either sequesters transcription factors or is incorporated into the preinitiation complex and interferes with transcription at the gene promoter. Given that changes in gene expression occur over decades, a second hypothesis is that early changes in gene expression influence later changes in gene expression (Li et al. 2002; Zhai et al. 2005; Hogel et al. 2012). Altered gene expression also occurs in a subset of genes in the cortex, which shows less neurodegeneration than the CPu but whose function is altered in HD, and in peripheral blood where these changes may be monitored as biomarkers of disease progression (Halliday et al. 1998; Denovan-Wright and Robertson 2000; Borovecki et al. 2002; Lovrecic et al. 2009).

The type 1 cannabinoid receptor (CB$_1$, CNR1) and the dopamine (DA) receptor type 2 (D$_R$D$_2$, DRD2) are expressed at high levels in the basal ganglia relative to other brain regions (Gerfen et al. 1990; Mailleux and Vanderhaeghen 1992; Matyas et al. 2006; Pickel et al. 2006). CB$_1$ and D$_R$D$_2$ regulate neuronal activity in the CPu, limbic system, and other regions of the brain as key components of the larger endocannabinoid system (ECS) and dopaminergic system (DAS), respectively (reviewed in Gerdeman and Fernandez-Ruiz 2008; El Khoury et al. 2012). Decreased expression of CB$_1$ and D$_R$D$_2$ is one of the earliest and most reliable transcriptional changes that occur during HD pathogenesis (Richfield et al. 1991; Cha et al. 1998; Glass et al., 2004; Denovan-Wright and Robertson 2000). Given that the expression and activity of these receptors is dependent upon the tone of their respective systems, it is logical that the ligands, enzymes, and other receptors that make up the ECS and DAS would also be dysregulated during HD progression, either as a direct result of mHtt or as a secondary response to changes in CB$_1$ or D$_R$D$_2$. For this reason, novel therapies that target ECS have generated significant interest as potential treatments for HD and existing therapies that target the DAS are currently being used to treat HD (Curtis et al. 2009; Dowie et al. 2009, 2010a,b; Blázquez et al. 2011; Sagredo et al. 2012; Pidgeon and Rickards 2013).

The ECS regulates neurotransmission and cell excitability throughout the central nervous system (CNS) and regulates cellular metabolism and migration in nonneuronal cells (reviewed in Howlett et al. 2002; Pertwee et al. 2010). In the brain, presynaptic CB$_1$ receptors are activated by endogenous (endo) cannabinoids, which are synthesized and released from the postsynaptic neuron following depolarization (Giuffrida et al. 2001; Kreitzer and Regher 2001). In the CNS, the type 2 cannabinoid receptor (CB$_2$, CNR2) is predominantly expressed in glial cells and is thought to inhibit proinflammatory processes (Atwood and Mackie, 2010; Lopez-Rodriguez et al. 2013). In addition to CB$_1$ and CB$_2$, the ECS is made up of putative cannabinoid receptors GPR18, and GPR119, the cation-permeable transient receptor potential vanilloid 1 (TRPV1), the anabolic enzymes diacyl glycerol lipase (DAGL), and N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), the catabolic enzymes abhydrolase domain-containing protein 4 (ABDH4), 6 (ABDH6), 12 (ABDH12), fatty acid amide hydrolase (FAAH), monoacyl glycerol lipase (MGLL), N-acylethanolamine acid amidase (NAAA), and the phosphatases Protein tyrosine phosphatase, nonreceptor type 22 (PTPN22) and Glycerophosphodiester phosphodiesterase 1 (GDE1), and the endocannabinoids (reviewed in Howlett et al. 2002; Pertwee et al. 2010). The most abundant endocannabinoids are anandamide (AEA) and 2-arachidonyl glycerol (2-AG; Martin et al. 1999). Endocannabinoids activate the nuclear receptor peroxisome proliferator-activated

receptor α (PPARα), which mediates many of the biological effects of cannabinoids including anti-inflammatory actions, feeding behavior, and analgesia (O'Sullivan 2007). Endocannabinoid levels can be regulated by cyclooxygenase 2 (COX-2, *PTGS2*) during inflammation (Hermanson et al. 2013). Finally, strong evidence suggests that another putative cannabinoid receptor, GPR55, is also part of the ECS (Henstridge 2012).

Dopamine is involved in the regulation of motor activity and coordination, cognition, motivation and emotion in the basal ganglia, as well as in many other regions of the brain (Nieoullon 2002; Cools 2008). In this study, we defined the DAS is the widest possible sense, including the G protein-coupled dopamine receptors types 1–5 (D_RD_{1-5}), anabolic enzymes in the DA synthesis pathway such as phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and DOPA decarboxylase (DDC), catabolic enzymes such as monoamine oxidase A (MAOA) and B (MAOB), and catechol-O-methyl transferase (COMT), and membrane channels such as the vesicular monoamine transporter (VMAT2, *SLC18A2*) and the dopamine transporter (DAT, *SLC6A3*; reviewed in Smith and Villalba 2008). Noradrenergic and adrenergic neurons, but not dopaminergic neurons, contain the enzyme dopamine β-hydroxylase (DBH) that converts DA to norepinephrine. Finally, the dopamine and cAMP-regulated phosphoprotein, 32 kDa (DARPP-32, *PPP1R1B*) is activated via $D_RD_{1/2}$-mediated cAMP accumulation and is known to be downregulated in the medium spiny projection neurons of HD patients (Bibb et al. 2000; van Dellen et al. 2000; Spires et al. 2004). Together these proteins represent the regulators, effectors, and transducers of information for the DAS.

The purpose of this study was to determine which changes in the ECS and DAS have been consistently observed over multiple microarray studies of HD progression using a meta-analytical approach. As with all systematic analyses, this study used defined criteria to investigate, collate, and assess a large number of independent studies in order that the data might be assembled to generate novel perspectives and hypotheses. The methods used employed a mathematical algorithm to collate biological data across multiple quantitative studies (Kalathur et al. 2012). Using this weighted sums approach we were able to estimate effect size (Kalathur et al. 2012). The approach resembled a random effects model of meta-analysis, which is a model commonly applied to heterogeneous data. Our aims were (1) to determine which changes in gene expression were consistently observed in independent studies; (2) to determine whether changes in gene expression occurred consistently in different models of HD; (3) to compare the patterns of gene expression in mouse and in vitro models of HD to changes observed in

human tissue; (4) to determine how changes in gene expression in the CNS compared to that of the periphery; and (5) to determine the temporal pattern of gene expression during disease progression. To do this, microarray data were collected from the Gene Expression Omnibus (GEO), Gene Expression Omnibus Datasets (GEO Datasets), Gene Expression Omnibus Profiles (GEO Profiles). Profiles database. Ultimately, our goal was to relate the systems-wide changes in gene expression that occur in the ECS and DAS during HD progression to the potential therapeutic targeting of the ECS and DAS for HD.

Materials and Methods

Databases, search pipeline, exclusion and inclusion criteria

We conducted a meta-analysis of differential gene expression in the ECS and DAS, using published microarray data, according to the guidelines described in (Kalathur et al. 2012). The components of the ECS and DAS were chosen based on existing literature and are described in Table 1. Computerized literature searches were conducted across the following four databases: MED-LINE, EMBASE, PubMed, and the Cochrane Central Register of Controlled Trials. To ensure that the widest possible range of studies were included, the search terms used were: "Huntington*" and "*array," "gene expression," or "differential*" (Pidgeon and Rickards 2013). The preliminary search generated 204 results and was subsequently limited to English language papers published after 2000. Further exclusion criteria included reviews, editorials, commentaries, and letters to the editors of scientific journals. We applied the following additional exclusion criteria to ensure only relevant studies were included in the analysis: studies using nongenetic models of HD (e.g., lesion models), studies solely examining epigenetic changes or changes in the expression of noncoding RNAs, and studies that did not comply with the MIAME standards (Brazma et al. 2001; Spellman et al. 2002; Kalathur et al. 2012). Following the application of exclusion criteria, and removal of unrelated and duplicate papers, 39 results remained (Table 2). Datasets from six additional studies were excluded from our results because they were not available through publicly available sources. Qualitative post-hoc comparisons were made between the findings of these studies and the datasets included in our quantitative meta-analysis (Luthi-Carter et al. 2000; Chan et al. 2002; Glass et al. 2004; Kuhn et al. 2007; Benn et al. 2010; Dowie et al. 2010b).

For each of the 39 results obtained (Table 2), the publicly available microarray data from each study were collected from the GEO using the GEO DataSets and

Table 1. Genes examined in this work.

Endocannabinoid system		Dopaminergic system	
Gene symbol	Gene name	Gene Symbol	Gene name
ABDH4	Abhydrolase domain-containing Protein 4	COMT	Catechol-o-methyl transferase
ABDH6	Abhydrolase domain-containing protein 6	DBH	Dopamine β-hydroxylase
ABDH12	Abhydrolase domain-containing protein 12	DDC	DOPA decarboxylase
DAGL	Diacyl glycerol lipase	DRD1	Dopamine receptor type 1
CNR1	Cannabinoid receptor type 1	DRD2	Dopamine receptor type 2
CNR2	Cannabinoid receptor type 2	DRD3	Dopamine receptor type 3
FAAH	Fatty acid amide hydrolase	DRD4	Dopamine receptor type 4
GDE1	Glycerophosphodiester phosphodiesterase 1	DRD5	Dopamine receptor type 5
GPR55	G protein-coupled receptor 55	MAOA	Monoamine oxidase A
GPR119	G protein-coupled receptor 119	MAOB	Monoamine oxidase B
GPR18	G protein-coupled receptor 199	PAH	Phenylalanine hydroxylase
MGLL	Monoacyl glycerol lipase	SLC6A3	Dopamine transporter (DAT)
NAPEPLD	*N*-acyl phosphatidylethanolamine phospholipase D	SLC8A2	Dopamine transporter (VMAT2)
NAAA	N-acylethanolamine acid amidase	TH	Tyrosine hydroxylase
PPARA	Peroxisome proliferator-activated receptor α	PPP1R1B	Dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32)
PTGS2	Prostaglandin-endoperoxide synthase 2, also known as cyclooxygenase-2 (COX-2)		
PTPN22	Protein tyrosine phosphatase, nonreceptor type 22		
TRPV1	Transient receptor potential cation channel subfamily V member 1		

Profiles tools. Data from two studies were unavailable through GEO and were obtained directly for the manuscripts (Luthi-Carter et al. 2002; Desplats et al. 2006). The search term used in GEO DataSets and GEO Profiles was "Huntington*." Filters were added to the search result in GEO Profiles by providing the gene symbol for each gene searched (Table 2) in *Homo sapiens and Mus musculus*, but no filters were placed on organism. GEO Profiles data were searched with the filter "Up/down genes" turned on *and* off to ensure no datasets were missed. All other filters and settings were left on default. The resulting GEO Profiles were then reviewed by hand to ensure they matched the studies collected in the literature search. Expression data for each gene were downloaded from GEO Profiles and subsequently used for analysis and scoring.

Analysis, scoring, and graphical output of the observed change in gene expression

Expression values for each dataset were imported into Microsoft Excel (Microsoft, 2008), organized by gene, and the mean and SEM for each gene for each group within a dataset was calculated. Significance between groups within a dataset was tested via one-way ANOVA followed by Tukey's post-hoc test using GraphPad (v. 5.0, Prism). If no significant difference between groups was found, then that dataset was assigned a value of 0. If a significant difference was found, then that dataset was assigned a

ranked value between 1 and 5 according to the model used in each dataset, where 1 was assigned for knockout mice that modeled HD but did not express mHtt, 2 for in vitro models of HD, 3 for rodent models of HD, 4 for stem cells derived from HD tissue, and 5 for human HD tissue samples (Kalathur et al. 2012). This ranking scheme was adopted and modified from Kalathur et al. (2012), who developed it in their study of changes in signal transduction that occur during HD progression as determined by analysis of data available on the HD research crossroads database (Kalathur et al. 2012). We chose to adopt the ranking system of Kalathur et al. (2012) because it is based on phenotypic and/or genetic similarity to the pathophysiology and molecular pathology observed in HD patients. Consequently, human tissue was given the highest value (5) and stem cells derived from patients with HD were given the second highest value (4). The ranked score was then given a positive or negative value if the change in gene expression was increased or decreased, respectively. The sum of the ranked scores from each dataset for each gene was then calculated according to the analysis being done. Sums were then normalized by dividing the number of studies that reported expression for that gene to arrive at the observed change in gene expression (OCGE). For example, the OCGE for *CNR1* shown in Figure 1A was determined by dividing the sum of the ranked values for all studies that reported the levels of *CNR1* transcripts (−63) by the number of studies (27 studies) to arrive at an OCGE of

Table 2. Studies included in this work.

| Species	Model	Region	Stage*	Systems analyzed	Reference	GEO accession
H. sapiens	HD donor tissue	CPu, Cb, PfACtx, MCtx	Pre-, Early, Middle, Late	ECS	Hodges et al. ((2006)	GSE3790
		CPu	Middle	ECS	Hu et al. (2011)	GSE24250
	HD patients	Peripheral blood	Pre-, Early	ECS and DAS	Borovecki et al. (2002)	GSE1767
		Peripheral blood	Middle	ECS and DAS	Runne et al. (2007)	GSE8762
		Peripheral blood	Pre-, Early, Middle, Late	ECS	Lovrecic et al. (2009)	GSE1751
	ESC	Stem cells	In vitro	ECS	Feyeux et al. (2012)	GSE34201
	iPSC	Stem cells	In vitro	ECS	An et al. (2012)	GSE37547
	PC12	Immortalized cell line	In vitro	ECS	van Roon-Mom et al. (2008)	GSE10581
M. musculus	R6/1	Str, Cb	Pre-, Early, Middle	ECS	Benn et al. (2005)	GSE3248
		Str	Middle	ECS	Desplats et al. (2006)	None
		Whole-brain	Early	ECS and DAS	Hodges et al. (2008)	GSE3621
	R6/2	Str	Early	ECS	Benn et al. (2005)	GSE3248
		Whole-brain	Middle	ECS	Morton et al. (2005)	GSE857
		Str	Middle	ECS	Thomas et al. (2011)	GSE857
	N171-82Q	Whole-brain	Middle	ECS	Luthi-Carter et al. (2002)	None
		Hpc, Cb	Early	ECS	Valor et al. (2013)	GSE44306, GSE44855, GSE44854
		Hpc, Cb	Early	ECS	Lopez-Atalaya et al. (2013)	GSE44868
	YAC128	Str	Pre-, Early, Middle	ECS	Morton et al. (2005)	GSE857
		Str	Early, Middle	ECS	Becanovic et al. (2010)	GSE18551, GSE19676, GSE19677
		Str	Middle	ECS	McConoughey et al. (2010)	GSE21237
	Hdh$^{Q111/Q111}$	Str	Middle	ECS	Horsch (2011)	GSE28232
		Str, Cb	In vitro	ECS and DAS	Fossale et al. (2011)	GSE9038
	CHL2(Q150/Q150)	Str	Pre-, Early, Middle	ECS	Morton et al. (2005)	GSE857
	Hdh$^{Q92/Q92}$	Str	Pre-, Early, Middle	ECS	Morton et al. (2005)	GSE857
	D9-N171-98Q	Str	Middle	ECS	Thomas et al. (2011)	GSE25232, GSE26317
	PGC1a$^{-/-}$ Hdh$^{Q150/Q150}$	Str	Middle, Late	ECS and DAS	Cui et al. (2006)	GSE5786
	Ctip2$^{-/-}$	Str	Middle, Late	ECS and DAS	Arlotta et al. (2008)	GSE9330
	Hdh$^{Q7/Q20}$	ESC	In vitro	ECS	Jacobsen et al. (2011)	GSE26001
	Hdh$^{Q7/Q50}$	ESC	In vitro	ECS		GSE26001
	Hdh$^{Q7/Q91}$	ESC	In vitro	ECS		GSE26001
	Hdh$^{Q7/Q111}$	ESC	In vitro	ECS		GSE26001
	STHdh$^{Q111/Q111}$	Immortalized cell line	In vitro	ECS and DAS	Lee et al. (2007)	GSE9025, GSE19780
		Immortalized cell line	In vitro	ECS	Riva et al. (2012)	GSE38000, GSE38001
	STHdh$^{Q109/Q109}$	Immortalized cell line	In vitro	ECS	Soldati et al. (2013)	GSE42107
	htt-171-82Q	Immortalized cell line	In vitro	ECS and DAS	Runne et al. (2008)	GSE1918, GSE12481
		Immortalized cell line	In vitro	ECS	Martin et al. (2012)	GSE39586
R. norvegicus	Transgenic Q51	Whole brain	Pre-, Middle	ECS	Nguyen et al. (2008)	GSE3790
	ST14A	Immortalized cell line	In vitro	ECS	Thompson et al. (2013)	GSE49392
S. cereviseae	Yeast		In vitro	ECS	Tauber et al. (2011)	GSE18644

*Pre-, Early, Middle, and Late refer to relative disease stage based on progression of motor dyskinesia in human patients with HD or animal models. For this study in vitro models were categorized as "Early." Cb, cerebellum; CPu, caudate and putamen; DAS, dopamine system; ECS, endocannabinoid system; ESC, embryonic stem cells; Hpc, hippocampus; iPSC, induced pluripotent stem cells; PfACtx, prefrontal association cortex; MCtx, motor cortex; Str, striatum.

−4.2. The error around the OCGE was determined by calculating the normalized sum of the ranked values for studies that *did not* report expression levels for a given gene (i.e., the upper and lower limit for the OCGE if all studies we examined had reported expression levels for a given gene). Thus, in Figure 1A, 12 studies did not include data on *CNR1* levels and the sum of ranked scores for these studies was 33; therefore, the error was ±2.75. The resulting OCGEs were analyzed using a one-sample t-test where x was the OCGE for a particular gene, μ_0 was set to 0, s was the standard deviation for a particular gene, and n was the number of reports for a

particular gene (GraphPad Prism v. 5.0). Post-hoc Holm-Bonferroni corrections for multiple testing were conducted for all datasets because multiple genes from multiple datasets were compared in these analyses (GraphPad Prism v. 5.0).

Observed change in gene expression for overall data in the CNS and peripheral blood, for the ECS and DAS, were graphed on modified Forest plots (Figs. 1A, B and 2A, B; GraphPad Prism v. 5.0). CNS data were not subdivided according to brain region. Significance was indicated with open squares. Closed squares indicate no significant difference. OCGEs for data grouped by disease
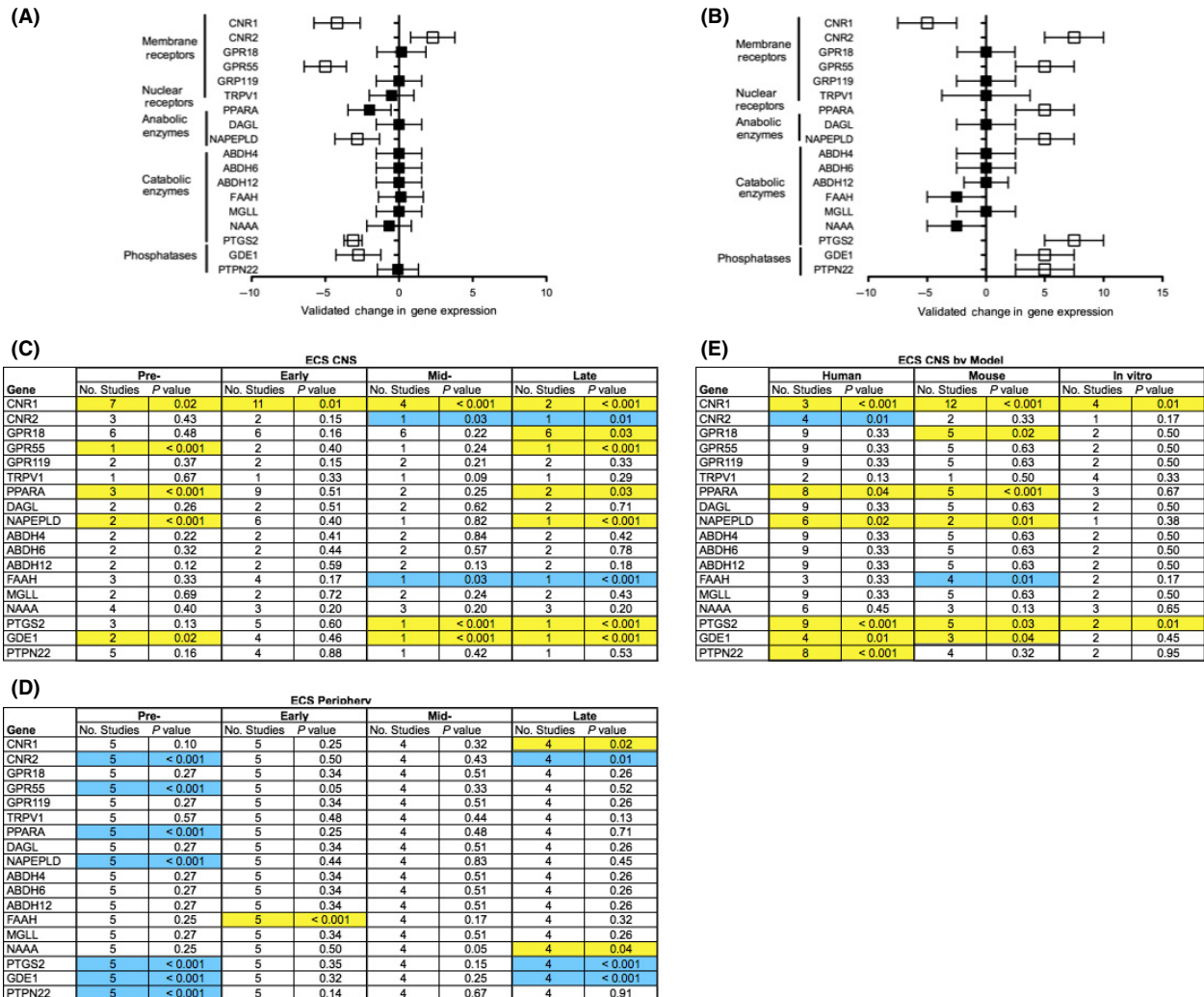
Figure 1. Transcriptional dysregulation of the ECS in microarray studies of HD. (A, B) Data are represented as the OCGE, which was the sum of the scoring matrix for each gene divided by the number of reports used in to generate that score ± the sum of the scoring matrix for all studies that did not report a change in the expression of each gene. Open boxes denote an OCGE different from 0 ($P < 0.05$). (A) Overall changes in gene expression in the CNS. (B) Overall changes in gene expression in the periphery. C–E) Significant OCGE relative to 0 are indicated by color. White: no change; yellow: decreased; light blue: increased. (C) Changes in gene expression in the CNS described according to disease stage. (D) Changes in gene expression in the periphery described according to disease stage. (E) Changes in gene expression in the CNS described according to model.

stage (Figs. 1C, D and 2C, D) or model (Fig. 1E), were represented as tables (Microsoft Excel, 2008). Disease stage was chosen based on motor symptom severity as described in each study. For the purpose of this study, in vitro data were described as "early." Significance (P value) and the number of studies used in each analysis are indicated for each gene. Color indicates the direction of change for each gene such that white indicates no change; yellow indicates a decrease; and blue indicates an increase. Details of the expression of different genes in each of the studies analyzed are provided in Tables S1 (ECS) and S2 (DAS).

Results

A meta-analytical approach was used to assemble and evaluate publicly available HD microarray datasets among microarray studies and compare these data to the previously published alternative molecular approaches that quantified changes in mRNA levels. Using defined inclusion criteria, 39 microarray studies assessing ECS gene expression, and 6 microarray studies assessing DAS gene expression were chosen for meta-analyses (Table 2).

The ECS within the CNS

In the CNS, the OCGEs for *CNR1, GPR55, NAPEPLD, PTGS2,* and *GDE1* were less than 0 ($P < 0.05$, Fig. 1A). The OCGE for *CNR2* was greater than 0 ($P < 0.05$, Fig. 1A). The OCGEs for *GPR18, GRP119, TRPV1, PPARA, DAGL, ABDH4, ABDH6, ABDH12, FAAH, MGLL,* and *NAAA* were not different from 0 (Fig. 1A). CNS microarray data were further divided according to the stage of the disease into "pre-symptomatic," "early," "middle," and "late" stage according to the description of motor symptom severity for the HD model used provided in the manuscript corresponding to each dataset (Fig. 1C). In vitro models were categorized as "early." The OCGE was less than 0 during the presymptomatic ($P < 0.05$), early ($P < 0.01$), middle ($P < 0.001$), and late ($P < 0.001$) stages for *CNR1;* presymptomatic ($P < 0.05$), middle ($P < 0.001$), and late ($P < 0.001$) stages for *GDE1;* early, middle, and late stages for *PTGS2* ($P < 0.001$); presymptomatic and late stages for *GPR55* ($P < 0.001$), *PPARA* ($P < 0.05$), and *NAPEPLD* ($P < 0.001$); and middle stage for *GPR18* ($P < 0.05$) (Fig. 1C). Further, the OCGE was greater than 0 during the middle ($P < 0.05$) and late ($P < 0.01$) stages for *CNR2* and *FAAH* (Fig. 1C).

To identify changes that occur in mouse and in vitro models of HD that recapitulate changes observed in human HD patients, we also conducted a comparison of OCGEs among human datasets, mouse models of HD, and in vitro models of HD for the ECS (Fig. 1E). OCGEs were consistently less than 0 for *CNR1* and *PTGS2* between human HD tissue, mouse, and in vitro models of HD (Fig. 1E). OCGEs were also less than 0 for *PPARA, NAPEPLD,* and *GDE1* between human HD tissue and mouse models of HD (Fig. 1E). OCGE values were greater than 0 for *CNR2* and less than 0 for *PTPN22* in human studies, but not mouse in vitro models of HD (Fig. 1E). Similarly, OCGE values were only greater than 0 for *FAAH* in mouse models of HD (Fig. 1E). Of all the changes in gene expression that have been reported, only change in *CNR1* and *PTGS2* consistently occurred in mouse and in vitro models of HD, but not others. Notably, the increase observed for *CNR2* is not observed consistently across studies, models of HD, and in human HD tissue.

The ECS in peripheral blood samples

Three microarrays studies reported on the expression of ECS transcripts in peripheral blood from patients with HD (Table 2). The samples taken from individuals with HD were staged as pre-symptomatic, early-, middle-, or late-symptomatic and compared with control samples from healthy individuals. These studies sought to identify biomarkers of HD onset and progression. We found that the OCGE for the pooled data was less than 0 for *CNR1,* and greater than 0 for *CNR2, GPR55, PPARA, NAPEPLD, PTGS2, GDE1,* and *PTPN22* ($P < 0.05$, Fig. 1B). The OCGEs were not different from 0 for *GPR18, GPR119, TRPV1, DAGL, ABDH4, ABDH6, ABDH12, FAAH, MGLL,* and *NAAA* (Fig. 1B). Changes in the levels of *CNR1* and *CNR2* have been extensively documented in the literature, whereas the changes in *GPR55, PPARA, NAPEPLD, PTGS2, GDE1,* and *PTPN22* have not been recognized as consistent in peripheral blood samples from HD patients. Further, when these data were compared with the overall data for the ECS in the CNS (Fig. 1A), *GPR55, NAPEPLD, PTGS2, GDE1,* and *PTPN22* OCGEs are all decreased in the CNS and increased in peripheral blood samples (Fig. 1B). Next, data were subdivided by disease stage. The OCGE was greater than 0 during the presymptomatic and late stages for *CNR2* ($P < 0.001$ and $P < 0.05$), *PTGS2* ($P < 0.001$), *GDE1* ($P < 0.001$); and the presymptomatic stage for *GPR55* ($P < 0.001$), *PPARA* ($P < 0.01$), *NAPEPLD* ($P < 0.001$), and *PTPN22* ($P < 0.001$) (Fig. 1D). The OCGE was greater than 0 during the early stage for *FAAH* ($P < 0.001$) and during the late stage for *CNR1* and *NAAA* ($P < 0.05$) (Fig. 1D). By dividing the data according to disease stage, we observed that all changes in gene expression in the peripheral ECS were stage specific. Moreover, the OCGE values for *FAAH* and *NAAA* were not different from 0 in the overall analysis, but were different at specific stages of HD. Therefore, several
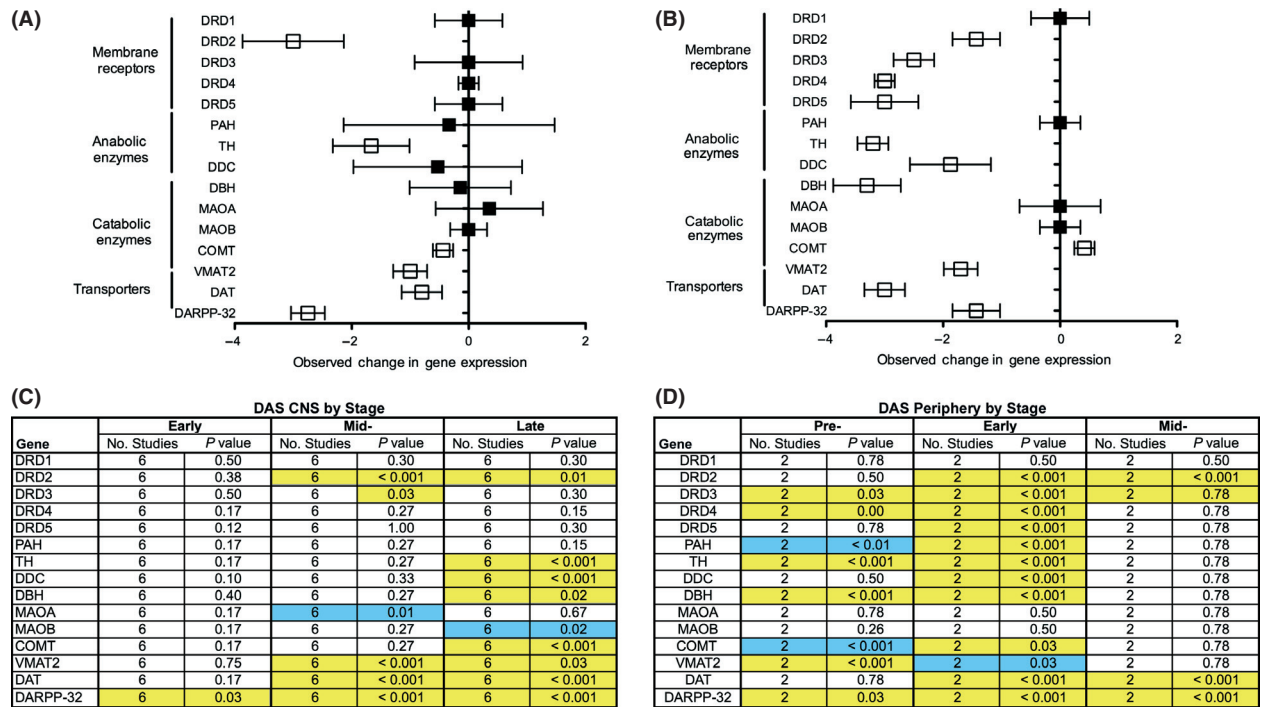
Figure 2. Transcriptional dysregulation of the DAS in microarray studies of HD. (A, B) Data are represented as the OCGE, which was the sum of the scoring matrix for each gene divided by the number of reports used in to generate that score ± the sum of the scoring matrix for all studies that did not report a change in the expression of each gene. Open boxes denote a OCGE different from 0 ($P < 0.05$). (A) Overall changes in gene expression in the CNS. (B) Overall changes in gene expression in the periphery. C, D) Significant OCGEs relative to 0 are indicated by color. White: no change; yellow: decreased; light blue: increased. (C) Changes in gene expression in the CNS described according to disease stage. (D) Changes in gene expression in the periphery described according to disease stage.

components of the ECS could be utilized as disease stage-specific biomarkers in HD.

The DAS within the CNS

We calculated the OCGE for each gene examined in the DAS in the CNS the six datasets that met our inclusion criteria (Table 2). We found that, in the CNS, the OCGEs for *DRD2*, *TH*, *COMT*, *VMAT2*, *DAT*, and *PPP1R1B* were less than 0 ($P < 0.05$, Fig. 2A). The OCGEs for *DRD1*, *DRD3*, *DRD4*, *DRD5*, *PAH*, *DDC*, *DBH*, *MAOA*, and *MAOB* were not different from 0 (Fig. 2A). CNS microarray data were further divided according to disease stage (Fig. 2C). In vitro models were categorized as "early." Only the OCGE for *PPP1R1B* was less than 0 during the early stage ($P < 0.05$) (Fig. 2C). The OCGE was less than 0 during the middle and late stages for *DRD2* ($P < 0.001$ and $P < 0.01$), *VMAT2* ($P < 0.01$ and $P < 0.05$), and *DAT* ($P < 0.01$), middle stage for *DRD3* ($P < 0.05$), and late stage for *TH* ($P < 0.01$), *DDC* ($P < 0.01$), *DBH* ($P < 0.05$), and *COMT* ($P < 0.01$) (Fig. 2C). Further, the OCGE was greater than 0 during the middle stage for *MAOA* ($P < 0.01$) and the late stage for *MAOB* ($P < 0.05$) (Fig. 2C). We could not

complete a comparison of models of HD for the DAS due to the limited number of studies that reported changes in the DAS. Importantly, we observed a disease stage-specific change in the OCGE for *DRD3*, *DDC*, *DBH*, *MAOA*, and *MAOB* where no difference had been observed in the overall analysis.

The DAS in peripheral blood samples

Two microarrays studies reported on the expression of DAS transcripts in peripheral blood from patients with HD (Table 2). Samples were taken from individuals with HD prior to motor symptom onset (presymptomatic), or in the early or middle stages of HD and compared with control samples from healthy individuals. The OCGE for the pooled data was less than 0 for *DRD2*, *DRD3*, *DRD4*, *DRD5*, *TH*, *DDC*, *VMAT2*, *DAT*, and *PPP1R1B*, and greater than 0 for *COMT* ($P < 0.05$, Fig. 2B). The OCGEs were not different from 0 for *DRD1*, *PAH*, *MAOA*, and *MAOB* (Fig. 2B). Next, data were then subdivided by disease stage. The OCGE was less than 0 during the presymptomatic, early, and middle disease stages for *DRD3* ($P < 0.05$ and $P < 0.001$); the presymptomatic and

early stages for *DRD4*, *TH*, and *DBH* ($P < 0.001$); the early stage for *DRD5* and *DDC* ($P < 0.001$); and the early and middle stages for *DRD2* and *DAT* ($P < 0.001$) (Fig. 2D). Intriguingly, the OCGE was greater than 0 during the presymptomatic stage and less than 0 during the early stage for *PAH* ($P < 0.01$) and *COMT* ($P < 0.001$ and $P < 0.05$) (Fig. 2D). The opposite was found for *VMAT2*, where the OCGE was less than 0 during the presymptomatic stage and greater than 0 during the early stage ($P < 0.001$ and $P < 0.05$) (Fig. 2D). The OCGE was less than 0 during all disease stages examined for *PPP1R1B* ($P < 0.05$ during the presymptomatic stage, $P < 0.001$ during the early and middle stages). Based on these data, the expression of several components of the DAS in peripheral blood samples, are changed in a disease stage-specific manner, which appears to be biphasic for *PAH*, *COMT*, and *VMAT2*.

Discussion

The main objective of this study was to determine the changes that were consistently observed in the ECS and DAS during HD progression both in the CNS and in peripheral blood in human HD tissue and different models of HD. We used a meta-analytical approach to examine independent studies using an unbiased mathematical approach. In this meta-analysis, publicly available microarray datasets from specific tissues, specific models, and distinct time points in HD progression were compared to the human condition. After the initial development of microarray technologies standards were put in place to provide publicly available curated microarray data (Allison et al. 2006). Such data appear in the GEO Profiles database. Within GEO Profiles investigators select subgroups of gene expression data to report; such that not every gene present on a microarray chip appears in the database. Within GEO Profiles we identified 39 microarray studies of the ECS, but only six microarray studies of the DAS included in this analysis. We did not find any publicly available microarray studies of human CNS tissue that examined the DAS.

The ECS in HD

Of all the changes that are reported in the various microarray studies, only changes in the expression of *CNR1*, *CNR2*, and *FAAH* have been consistently reported and investigated in previous studies (Luthi-Carter et al. 2000; Laprairie et al. 2013, 2014). Changes in the expression of *CNR1* have been reported in several HD mouse models using microarray in other studies that were not available in the GEO Profiles datasets (Luthi-Carter et al. 2000; Chan et al. 2002; Kuhn et al. 2007; Benn et al. 2010). Similarly, dysregulation of *CNR1* expression has been observed using

qRT-PCR and in situ hybridization (Denovan-Wright and Robertson 2000; Glass et al. 2000). Increased levels of *CNR2* and *FAAH* mRNA have been documented via qRT-PCR and western blot in R6/2 mouse models of HD, ST*Hdh*$^{Q111/Q111}$ cells, and tissue from HD patients (Blázquez et al. 2011; Bari et al. 2013; Laprairie et al. 2014). We observed increased FAAH levels in the late stages of HD, which may indicate increased AEA catabolism (Laprairie et al. 2014). Dysregulation of ECS tone may follow changes in the expression FAAH or other anabolic and catabolic cannabinoid enzymes (Bari et al. 2013).

The DAS in HD

Alterations in gene expression of components of the DAS in the basal ganglia have been linked to the pathology of HD in both postmortem human and animal models (El Khoury et al. 2012). Changes in the expression of *DRD2*, *TH*, *DAT*, *VMAT2*, and *PPP1R1B* (DARPP-32) have been consistently reported in previous studies (Bibb et al. 2000; Glass et al. 2000; Suzuki et al. 2001; Yohrling et al. 2003). Reduction in the expression of D_RD_2 is well documented in pre- and symptomatic human patients and R6/2 mice models of HD using in situ hybridization, qRT-PCR, autoradiographic, and positron emission tomography (Joyce et al. 1988; Richfield et al. 1991; Antonini et al. 1996; Augood et al., 1997; Glass et al. 2000; Bibb et al. 2000). Other microarray studies that did not provide publicly available data have also reported lower striatal D_RD_2 levels in several HD mouse models including: R6/2, R6/1, N171-82Q, YAC72, and Hdh$^{Q92/Q92}$ mice (Luthi-Carter et al. 2000; Chan et al. 2002; Kuhn et al. 2007; Benn et al. 2010). Postmortem studies of late-stage HD patients, R6/2, and YAC128 mice models of HD showed reduced levels of caudate DA and homovanillic acid, the principal DA metabolite (Bernheimer et al. 1973; Reynolds and Garrett 1986; Kish et al. 1987; Reynolds et al. 1999; Callahan and Abercrombie 2011). Changes in the expression of anabolic enzymes in the CNS that are involved in DA synthesis might play a role in the reduction of DA. The levels of TH, the enzyme that catalyzes the rate-limiting step in the biosynthesis of DA, have been demonstrated previously to decline during late stages of HD progression in HD patients and in R6/2 mice (Yohrling et al. 2003). The majority of synaptic DA is cleared by DAT and VMAT2. Similarly to the microarray studies, previous analysis of postmortem brains from late-stage HD patients has shown reduced striatal expression of DAT and VMAT2 using autoradiography (Suzuki et al. 2001). Reduction in DARPP-32 expression was previously observed in postmortem human HD and R6/2 mice using immunohistochemistry (Bibb et al. 2000; van Dellen et al. 2000).

Comparing changes in the ECS and DAS between mouse models of HD

The data presented here indicate that no single gene in the ECS was consistently up- or downregulated in all mouse models included in these analyses (Table S1). The most consistently observed change among ECS genes was downregulation of *CNR1* in 81% of studies utilizing mouse models of HD. *GPR18, GPR55,* and *PTGS2* levels were lower in 28% of studies, *PPARA* levels were lower in 21% of studies, *NAPEPLD* and *GDE1* levels were lower in 12% of studies, and *FAAH* levels were higher in 12% of studies (Table S1). Among genes analyzed in the DAS, *DRD2, DRD3, DAT,* and *PPP1R1B* were consistently downregulated in HD samples (Table S2). However, the limited size of this dataset means that these observations are difficult to interpret. Interstudy variability also existed between mouse models of HD. For example, *FAAH* was reported as upregulated in some studies of R6/1 mice (Benn et al. 2005; Desplats et al. 2006), but not others (Hodges et al. 2008). Similarly, *PTGS2* and *GDE1* were only downregulated in R6/1 mice and no other model examined. Some of the models used in these datasets may not have been old enough to display HD-like transcriptional dysregulation, as previous reports have suggested knock-in models of HD do not display transcriptional dysregulation until later in disease presentation (Kuhn et al. 2007). Similarly, the novel zQ175 knock-in mouse model of HD displays a subset of the transcriptional abnormalities observed in R6/1 and R6/2 mice, including reduced *CNR1* expression, during that time of motor symptom onset rather than prior to motor symptom onset (Menalled et al. 2012). Although no microarray analyses have been done with zQ175 mice, this mouse model exhibits metabolic, behavioral, and motor deficits over a longer time course than the rapidly degenerating R6 lines, suggesting this model may be most useful for the study of long-term behaviors and phenotypes (Menalled et al. 2012). These data demonstrate that variability in the pattern of transcriptional dysregulation exists between different mouse models of HD. While certain changes, such as decreased levels of *CNR1*, are relatively robust, other changes may be model specific and more readily detected in aggressive models of HD, such as R6/1 and R6/2 mice.

Comparing data from mouse models of HD to human tissue studies

One major use of these data is determining the predictive power of animal models of HD in the rational development of therapies for patients suffering from HD. Our analysis revealed that, in general, changes in *CNR1*,

PPARA, NAPEPLD, PTGS2, and *GDE1* were consistent between mouse and human studies. This comparative analysis is not possible for the DAS due to the limited size of the dataset. When individual mouse models are compared to the observations made in human tissue studies, all models examined (Table 2) reported the well-characterized downregulation of *CNR1*, but only the R6/1 and R6/2 mouse models, with some exceptions, displayed similar patterns of gene expression to those observed in human datasets (Table S1). The strong correlation between the genome-wide pattern of transcriptional dysregulation in R6 mouse models of HD and human tissue samples has been noted elsewhere (Kuhn et al. 2007). The absence of correlation in our data between mouse models of HD and human HD tissue samples may be the result of the small number of genes selectively analyzed (Kuhn et al. 2007). Here, we demonstrate that the pattern of gene expression for the ECS is largely similar between R6 mouse models and samples from human HD tissue and largely dissimilar between other mouse models of HD compared to human HD tissue. It is important to note that while no single mouse model of HD can be considered the most valid tool for assessing disease progression or the efficacy of novel therapeutics. The animal model must be carefully chosen as the most suitable tool to answer the question being posed.

Changes in the ECS and DAS that occur in peripheral blood may be useful as biomarkers in HD

HD is often described as a neurodegenerative disease rather than a disease that affects the whole body. The data presented here demonstrate that changes in gene expression occur in the peripheral blood in a disease stage-specific manner to an equal or greater extent than in the CNS. It is important to note that while changes in gene expression in peripheral blood may not occur in the CNS, but can still be followed as biomarkers of disease progression. Although the pathological implications of these changes are unknown, novel stage-specific biomarkers for HD could improve the selection and efficacy of therapies for the treatment of HD (Runne et al. 2007).

Targeting the endocannabinoid and dopamine systems in the treatment of HD

There are currently no treatments available to cure or delay the progression of HD. Studies in animal and in vitro models of HD suggest that certain CB$_1$ agonists may reduce hyperkinetic movement in HD and promote the survival of medium spiny projection neurons (Dowie et al. 2009, 2010a,b; Blázquez et al. 2011). One clinical

trial using nabilone, a CB_1/CB_2 agonist, has reported minor, but significant, improvements in chorea and irritability in HD patients (Curtis et al. 2009). Another clinical trial exploring the beneficial effects of Sativex (THC and cannabidiol) for the treatment of chorea in HD has recently been completed although the results have not yet been published (National Institutes of Health). Two other therapeutic targets in the ECS are FAAH and CB_2. Because FAAH levels are elevated late in HD, FAAH inhibitors may be able to restore ECS functionality in HD via CB_1 (Bari et al. 2013; Laprairie et al. 2014). Therefore, several components of the ECS offer potential therapeutic targets for the treatment of HD.

Existing pharmacological treatments only alleviate motor and cognitive symptoms of HD (reviewed in Pidgeon and Rickards 2013). Treatments targeting the DAS have to be chosen according to the stage of the disease because DA levels are increased early in HD pathogenesis and decrease as the disease progresses (Chen et al. 2013). Clinical evidence also suggests that D_RD_2 antagonists, such as haloperidol, olanzapine, risperidone, quetiapine, and ziprasidone, might be beneficial to treat chorea, changes in mood, and behavior during early stage of HD (reviewed in Pidgeon and Rickards 2013). Targeting other components of the DAS for the treatment of HD has also been investigated. For example, tetrabenazine, the only drug approved for treatment of chorea in HD, acts as a VMAT inhibitor and D_RD_2 antagonist (Mestre and Ferreira 2012). During late stages of the disease drugs that increase DA levels, such as bromocriptine, lisuride. and aripiprazole, may alleviate akinesia (Mestre and Ferreira 2012). MAOA and MAOB expression is also elevated in the basal ganglia during the latter stages of HD (Richards et al. 2011). Elevation of DA via MAOB inhibition may, therefore, have beneficial effects during the late stages of HD (Pidgeon and Rickards 2013). However, contradictory clinical results have been reported for these drugs, which demonstrate the time-dependent changes that occur in the DAS as the disease progresses (Chen et al., 2013).

In situ hybridization studies assessing mRNA expression and immunohistochemistry studies assessing protein expression have shown that CB_1 and D_RD_2 are colocalized in the GABA-ergic medium spiny neurons projecting from the striatum to the globus pallidus, as well as on the axon terminals at the globus pallidus (Gerfen et al. 1990; Mailleux and Vanderhaeghen 1992; Hermann et al. 2002; Matyas et al. 2006; Pickel et al. 2006). Colocalization of these receptors in the basal ganglia may allow for bidirectional regulation between the ECS and DAS through the formation of functional heterodimers (Fernandez-Ruiz et al. 2010). D_RD_2 modulates release of AEA in the dorsal striatum (Giuffrida et al. 1999), and facilitates endocanna-binoid-mediated long-term synaptic depression of GABA-ergic neurons (Pan et al. 2008). In addition, receptor agonists may modulate subcellular localization, receptor expression and homo- and heterodimer ratios of CB_1 and D_RD_2 (Przybyla and Watts 2010). Given these interactions between the ECS and the DAS, drugs that effect one system will likely effect the other (Wiley et al. 2008), yet the consequences of cannabinoid treatment on dopaminergic tone or DA treatment on endocannabinoid tone are unknown in healthy individuals, let alone people suffering from HD. Moreover, the possible recreational or illicit use of *cannabis* by HD patients may alter the efficacy of drugs targeting the DAS. Therefore, a better understanding of the relationship between the ECS and DAS is not only important in and of itself, but it is also directly applicable to HD and the design of therapies for HD.

Conclusions

The consolidated data presented here provide a high-level summary of changes that occur in the ECS and DAS–two systems whose functionality is dysregulated in HD. These data provide a point of consensus for future studies where the ECS and DAS are targeted for the management of HD in different models, stages of disease progression, and in the human condition. This meta-analysis is also a useful reference for future microarray array work in HD. Finally, given the lack of DAS data available in GEO profiles, future studies should include DAS data in publicly accessible microarray datasets regardless of how these genes are affected in HD, and regardless of whether these genes are affected, particularly where preclinical therapies are tested in disease models.

Because HD affects gene expression in the ECS and DAS, the efficacy of drugs that target these systems may depend on the disease stage-specific tone of the system being targeted. Moreover, dopaminergic drugs and cannabinoids could affect the expression of their target genes, which may or may not be therapeutically desirable. But perhaps the greatest gap in our knowledge is the relationship between the ECS and DAS in the CNS of healthy individuals and patients suffering from HD. Characterization of the crosstalk that occurs between the ECS and DAS may lead to the development of novel therapeutics that exploit the connections and commonalities between these systems.

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Disclosures

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Detailed summary of OCGE values by study for the endocannabinoid system. GEO DataSets Accession Numbers are listed below each species. GEO Profiles data were used in data analyses. Significant OCGEs relative to 0 are indicated by color. White: no change; yellow: decreased; light blue: increased; black: no data.

Table S2. Detailed summary of OCGE values by study for the dopaminergic system.GEO DataSets Accession Numbers are listed below each species. GEO Profiles data were used in data analyses. Significant OCGEs relative to 0 are indicated by color. White: no change; yellow: decreased; light blue: increased; yellow and light blue: the study reports disease stage-specific changes in gene expression.