Measuring Disease Phenotype Reversal of HD Related Genomics Signatures in Response to Therapeutic and Genetic Perturbations Using Posterior Probabilities

Jonathan R. Greene[1], Christopher Hartl[1], John C. Obenauer[1], Jeffrey S. Aaronson[2], Ramee Lee[2], Deanna Marchionini[2], Vahri Beaumont[2], Tom Vogt[2], Ignacio Munoz-Sanjuan[2], Jian Chen[2], Jim Rosinski[2]

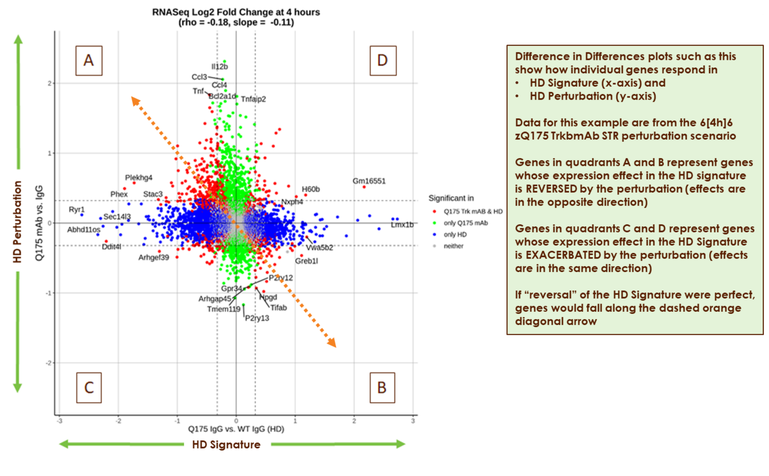
[1]Rancho BioSciences LLC, San Diego, CA; [2]CHDI Management/CHDI Foundation, Princeton, NJ

Abstract

There is great interest in genomics studies involving therapeutic and genetic perturbations in HD model systems. A common question that arises in these studies is the extent to which a given therapeutic or genetic perturbation can modulate (reverse, prevent, or exacerbate) transcript or protein level effects of the HD model at the broad global level, biological pathway level, as well as the level of individual genes or proteins. We describe here a method for making such determinations based on individual feature (eg gene or protein) expression statistics derived from differential expression analysis. The method involves defining a broad "HD Signature" list of features that are affected in the HD model system absent any perturbation and then for each feature determining the posterior probability that the feature displays a given level of modulation in the treated HD model. The directionality and magnitude of the effect is then used to classify these modulations and the individual probabilities are rolled up to define overall modulation percentages. Along with describing this methodology in detail, we will present examples from relevant studies.

Description of the Method

- Studies subject to this analysis method involve an HD model system and perturbations involving genetic modifications or molecular agents
  - e.g, those known to or are suspected of lowering levels of mutant Huntingtin
- The analysis paradigm is based on two differential expression analysis measurements, such as those performed using R packages such as DESeq2 or limma
  - HD Signature contrast: HD Model vs. Wild type, both un-perturbed
  - HD Perturbation contrast: Perturbed HD Model vs. un-Perturbed HD Model
  - In addition, a Perturbed Wild Type vs. un-Perturbed Wild Type contrast can be included to filter out "background" effects that are less likely to be relevant to HD



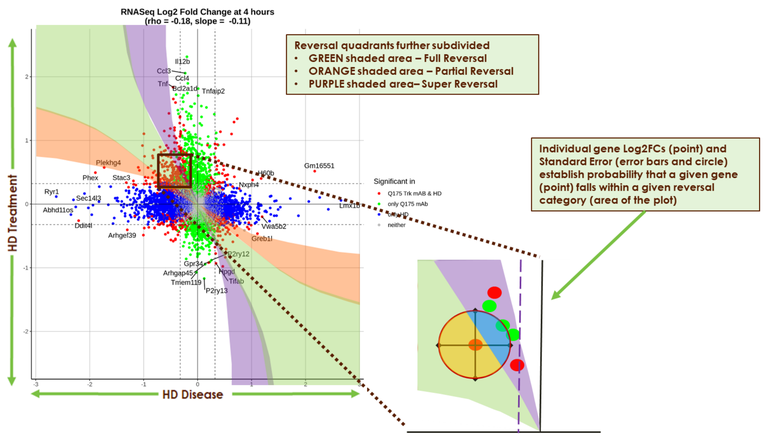
Difference in Differences plots such as this show how individual genes respond in
- **HD Signature (x-axis) and**
- **HD Perturbation (y-axis)**

Data for this example are from the 6[4h]6 zQ175 TrkbmAb STR perturbation scenario

Genes in quadrants A and B represent genes whose expression effect in the HD signature is REVERSED by the perturbation (effects are in the opposite direction)

Genes in quadrants C and D represent genes whose expression effect in the HD Signature is EXACERBATED by the perturbation (effects are in the same direction)

If "reversal" of the HD Signature were perfect, genes would fall along the dashed orange diagonal arrow



Reversal quadrants further subdivided
- **GREEN shaded area – Full Reversal**
- **ORANGE shaded area – Partial Reversal**
- **PURPLE shaded area– Super Reversal**

Individual gene Log2FCs (point) and Standard Error (error bars and circle) establish probability that a given gene (point) falls within a given reversal category (area of the plot)

Define HD Signature from HD Disease comparison
- EG: Abs(FC) > 1.15 and adjusted p-value < 0.05
- These parameters are flexible
- Performance of the method overall relies on a robust HD Signature

Define HD Treatment modulation classes
- Full reversal
- Partial reversal
- Super reversal
- Exacerbation

Determine each gene's individual posterior probability for each class

Sum up to determine overall reversal probability

Reversal genes have an overall reversal probability > 0.95

Assign reversal category to that with the highest probability

Description of the Method

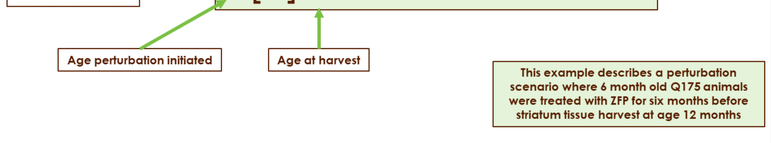
- The posterior-probability based reversal method ("PP" for short) attempts to quantify the probability that the treatment effect reverses, either partially or fully, the disease effect on a gene by gene basis. This is done by viewing the treatment effect as a multiple of the disease effect (in log-fold-change), i.e., Δ_treat=αΔ_disease. If $\alpha < 0$, then the disease effect is reversed by the treatment; while if $\alpha > 0$, the disease effect is exacerbated by treatment.
- The PP method examines 5 possible cases for α, for example:
  - Super-reversal: $\alpha < -1.3$
  - Full reversal: $-1.3 < \alpha < -0.7$
  - Partial reversal: $-0.7 < \alpha < -0.2$
  - Negligible reversal: $-0.2 < \alpha < 0.2$
  - Exacerbation: $\alpha > 0.2$
- Once the probabilities for each region are calculated, a gene is assigned to a reversal category by the following logic
  - Probabilities for full reversal, partial reversal, and super reversal are summed to determine an Overall Reversal Probability
  - If P[Overall Reversal] > 0.95, the gene is considered to be reversed
  - Reversed gene is assigned to the reversal category with the highest probability
- Dependent on when the perturbation was initiated in the HD model, a "reversal" could chronologically represent a *prevention* of the gene dysregulation rather than a true reversal of a differentially expressed gene

HD Model Studies

Study	Brief Description	HD Signature Contrast	HD Perturbation Contrast
Q175 nZFP	Zinc finger repressor protein directed towards CAG repeats, known to selectively lower levels of mHtt protein, driven by human synapsin promoter for neuronal expression, delivered by AAV[1] Control agent is AAV ZFP construct lacking the DNA Binding Domain (DBD) 6 month old Wild Type or Htt Q175$^{+/-}$ mice are treated with nZFP or DBD for 1 or 6 months	Q175 DBD treatment vs WT DBD treatment	Q175 nZFP treatment vs Q175 DBD treatment
Q175 TrkmAb	Monoclonal antibody agonist 38B8 targeting the neurotrophic receptor tyrosine kinase 2[2] Control agent is IgG 6 month old Wild Type or Htt Q175$^{+/-}$ mice are acutely (4h or 24h) treated with TrkB mAb or IgG	Q175 IgG treatment vs WT IgG treatment	Q175 TrkB treatment vs Q175 IgG treatment
Q175 Pde10a inhibitor	NCBI GEO Study GSE89505, Pde10a inhibitor PF_02545290[3] Wild Type or Htt Q175$^{+/+}$ mice are treated with Pde10a Inhibitor or Vehicle po qd from 5 weeks to 9 months	Q175 Vehicle vs WT Vehicle	Q175 Pde10a Inhibitor vs Q175 Vehicle
LacQ140^{i}(*)	Expression of the Q140-*Htt* allele is regulated by transcriptional control elements derived from the E. coli Lac Operon and a Lac regulator repression (LacIR) transgene, under control of the b-actin promoter (*Tg*$^{ACTB\text{-}lacI*SceI}$ mice). Expression of *mHtt* is regulated with IPTG	LacQ140^{i}(*) exposed to IPTG since embryonic day 5 vs Wild Type exposed to IPTG since embryonic day 5	LacQ140^{i}(*) with IPTG removed from water prenatally or at 2 or 8 months vs LacQ140^{i}(*) exposed to IPTG since embryonic day 5

1. Zeitler B et al. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease. Nat Med. 2019 Jul;25(7):1131-1142.
2. Todd et al. A monoclonal antibody TrkB receptor agonist as a potential therapeutic for Huntington's disease. PLoS One. 2014 Feb 4;9(2):e87923.
3. Beaumont et al. Phosphodiesterase 10A Inhibition Improves Cortico-Basal Ganglia Function in Huntington's Disease Models. Neuron. 2016 Dec 21;92(6):1220-1237.

We use the following type of nomenclature to label HD "perturbation scenarios":



This example describes a perturbation scenario where 6 month old Q175 animals were treated with ZFP for six months before striatum tissue harvest at age 12 months

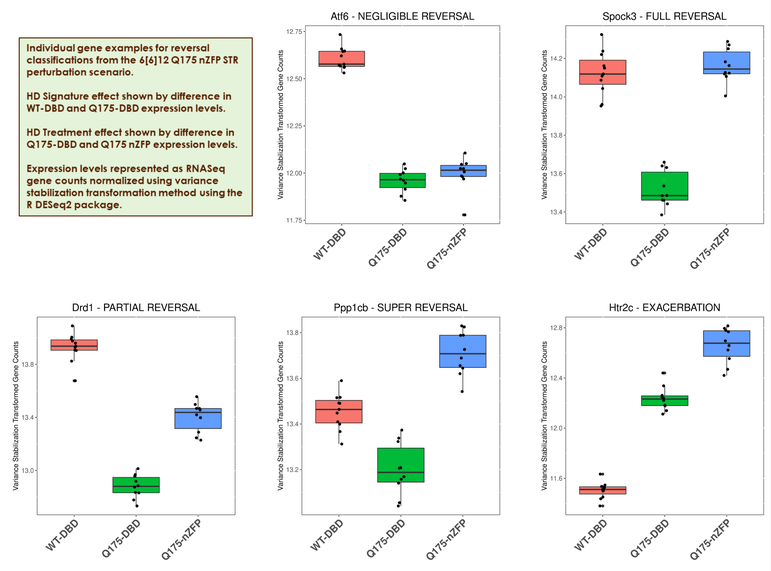
Results

Individual gene examples for reversal classifications from the 6[6]12 Q175 nZFP STR perturbation scenario.

HD Signature effect shown by difference in WT-DBD and Q175-DBD expression levels.

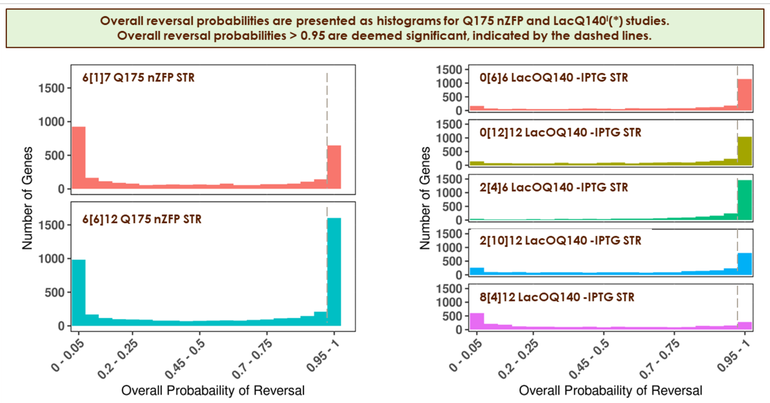
HD Treatment effect shown by difference in Q175-DBD and Q175 nZFP expression levels.

Expression levels represented as RNASeq gene counts normalized using variance stabilization transformation method using the R DESeq2 package.

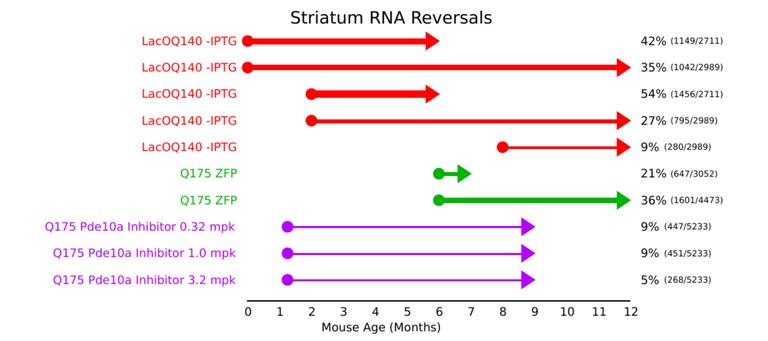


Results

Overall reversal probabilities are presented as histograms for Q175 nZFP and LacQ140^{i}(*) studies. Overall reversal probabilities > 0.95 are deemed significant, indicated by the dashed lines.



Percentage of reversed genes is presented along with numbers for HD Signature genes and genes that are overall reversed. Start and end points represent administration of perturbation and age of harvest.

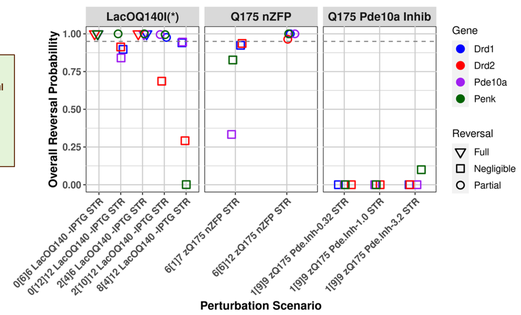


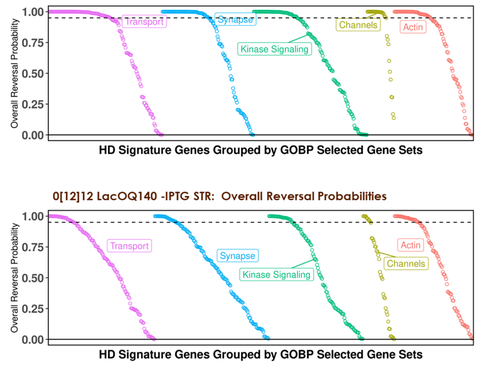
Reversal "Census" for multiple perturbation scenarios: counts for genes in HD Signature and various reversal categories.

Scenario	Study	HD Signature	Reversed	Pct. Reversed	Exacerbation	Pct. Exacerbated	Full Reversal	Partial Reversal	Negligible Reversal	Super Reversal
6[24h]6 zQ175 TrkbmAb STR	Q175 TrkmAb	3407	700	21	344	10	263	389	2363	48
6[4h]6 zQ175 TrkbmAb STR	Q175 TrkmAb	4079	762	19	342	8	303	360	2975	99
0[6]6 LacOQ140 -IPTG STR	LacQ140^{i}(*)	2711	1149	42	11	0	522	582	1551	45
0[12]12 LacOQ140 -IPTG STR	LacQ140^{i}(*)	2989	1042	35	4	0	248	786	1944	8
2[4]6 LacOQ140 -IPTG STR	LacQ140^{i}(*)	2711	1456	54	1	0	527	915	1254	14
2[10]12 LacOQ140 -IPTG STR	LacQ140^{i}(*)	2989	795	27	12	0	147	646	2182	2
8[4]12 LacOQ140 -IPTG STR	LacQ140^{i}(*)	2989	280	9	17	1	66	214	2692	0
6[1]7 Q175 nZFP STR	Q175 nZFP	3052	647	21	149	5	190	430	2256	27
6[6]12 Q175 nZFP STR	Q175 nZFP	4473	1601	36	232	5	566	954	2640	81
1[8]9 zQ175 Pde.Inh-0.32mpk STR	Q175 Pde10a Inhib	5233	447	9	216	4	81	364	4570	2
1[8]9 zQ175 Pde.Inh-1.0mpk STR	Q175 Pde10a Inhib	5232	451	9	270	5	119	323	4511	9
1[8]9 zQ175 Pde.Inh-3.2mpk STR	Q175 Pde10a Inhib	5231	268	5	139	3	81	151	4824	36

Reversal classifications and overall reversal probabilities for individual genes across multiple perturbation scenarios.

Overall reversal probabilities > 0.95 are considered significant.





HD Signature genes from GOBP gene sets generally relevant to HD signatures are plotted out by individual gene overall reversal probability.

Summary

- Reversal and exacerbation genes determined by posterior probabilities provide an interpretable summary statistic and gene filter within and between studies.
- Gene by gene statistics can be rolled up to level of overall signature, particular gene lists of interest, or genes represented in relevant pathways and gene sets.

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