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Genomic Analysis Reveals Disruption of Striatal Neuronal Development and Therapeutic Targets in Human Huntington's Disease Neural Stem Cells

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SUMMARY

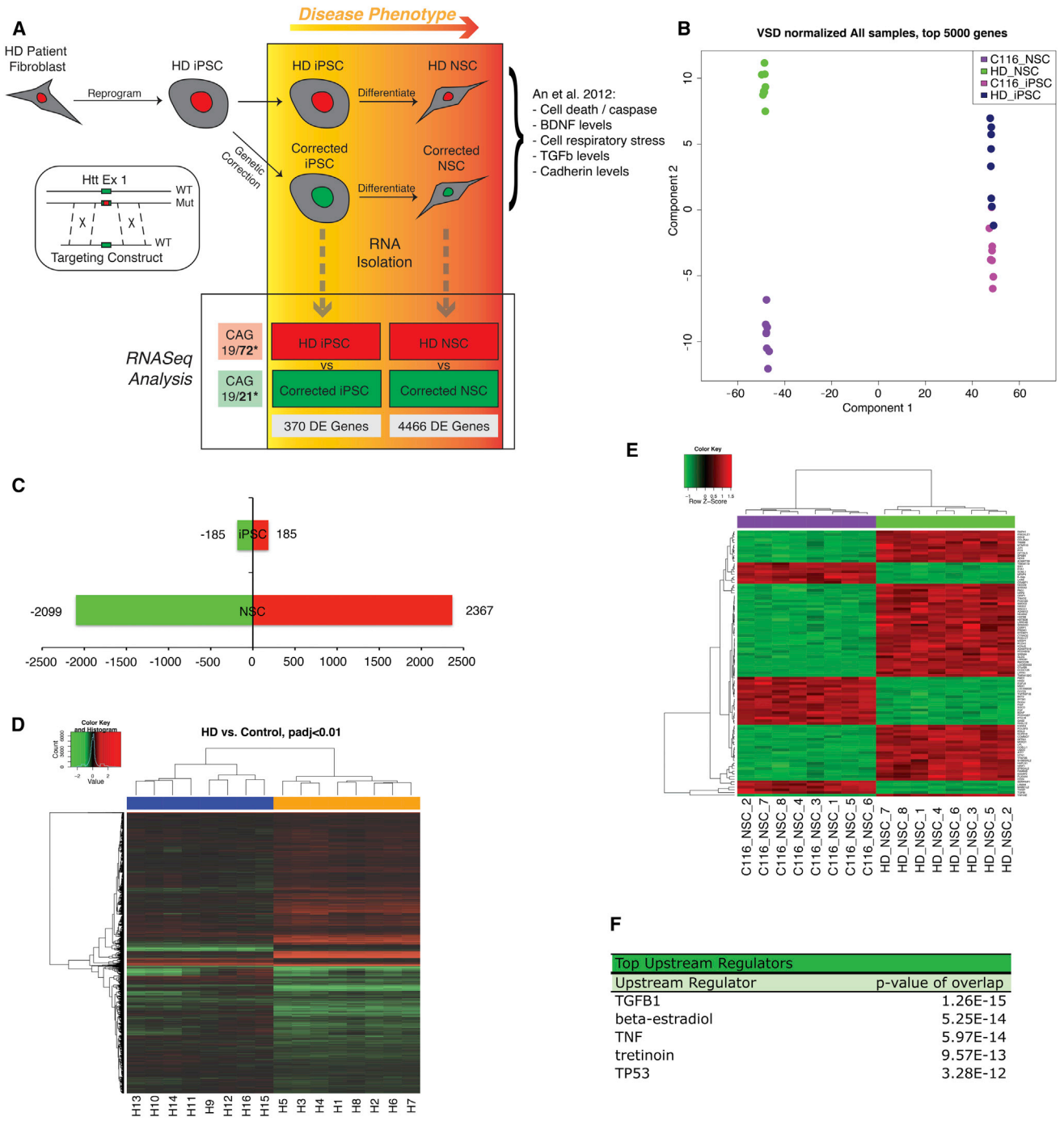
We utilized induced pluripotent stem cells (iPSCs) derived from Huntington's disease (HD) patients as a human model of HD and determined that the disease phenotypes only manifest in the differentiated neural stem cell (NSC) stage, not in iPSCs. To understand the molecular basis for the CAG repeat expansion-dependent disease phenotypes in NSCs, we performed transcriptomic analysis of HD iPSCs and HD NSCs compared to isogenic controls. Differential gene expression and pathway analysis pointed to transforming growth factor β (TGF-β) and netrin-1 as the top dysregulated pathways. Using data-driven gene coexpression network analysis, we identified seven distinct coexpression modules and focused on two that were correlated with changes in gene expression due to the CAG expansion. Our HD NSC model revealed the dysregulation of genes involved in neuronal development and the formation of the dorsal striatum. The striatal and neuronal networks disrupted could be modulated to correct HD phenotypes and provide therapeutic targets.

INTRODUCTION

Huntington's disease (HD) is a debilitating, inherited neurological disorder characterized by chorea, psychological changes, and cognitive decline leading to dementia (Victorson et al., 2014). These symptoms are correlated with loss of striatal and cortical neurons in the brain (Ehrlich, 2012). HD is caused by a CAG expansion mutation coding for the polyglutamine tract located in the N-terminal region of the huntingtin protein (HTT) (The Huntington's Disease Collaborative Research Group., 1993). At this time, there is no cure for HD or treatments to delay its onset and progression (Videnovic, 2013).

Standard HD models include transgenic animal models, immortalized rodent and human cell lines, and post-mortem tissue from HD patients (Bard et al., 2014; Lee et al., 2013). These models have been very useful in understanding some mechanisms behind HD pathogenesis; however, they do not fully represent human HD pathology (Kaye and Finkbeiner, 2013). Particularly important is the field's reliance on HD mouse models, which do not account for the potential to miss key drug targets, the effects of polymorphisms on human protein toxicity, human-specific cell subtypes, and transcription factor binding sites specific to humans. A promising complementary approach for modeling HD is the use of human induced pluripotent stem cells (iPSCs) derived from HD patient somatic cells (An et al., 2012; Zhang et al., 2010). HD iPSCs harboring mutant HTT protein (mHTT) have the potential to model the disease more accurately, as they are untransformed and capable of differentiating into multiple types of neural tissue. Human iPSCs also provide the advantage of following the progress of the disease during neural development and detecting early pathological changes—the presymptomatic stage. iPSCs provide a platform for systemic genomic profiling and drug screening and are a promising tool for cellular replacement therapy in HD patients.

We have previously established HD-patient-derived iPSCs and corrected their genetic defect through the use of homologous recombination-based gene targeting methods (An et al., 2012). Characterization of these isogenic lines and derivative neural precursors showed that correction of the expanded polyglutamine region to a non-disease causing length resulted in a normalization of cellular phenotypes consistent with several well-established and reproducible aspects of the disease—cell death, loss of brain-derived neurotrophic factor (BDNF) expression, and reduction of mitochondrial respiratory capacity, among other cellular phenotypes (An et al., 2012; Zhang et al., 2010). These phenotypes were apparent in differentiated neural stem cells (NSCs) but not the pluripotent stem cell fate. In a separate study, HD iPSCs displayed elevated lysosomal activity indicating a disruption in cellular maintenance and protein degradation (Camnasio et al., 2012). Finally, a study identified the key functional differences



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Top Upstream Regulators	
Upstream Regulator	p-value of overlap
TGFB1	1.26E-15
beta-estradiol	5.25E-14
TNF	5.97E-14
tretinoin	9.57E-13
TP53	3.28E-12

Figure 1. RNA-Seq Analysis of HD Isogenic Stem Cell Model

(A) Schematic illustration of experimental design and analysis. iPSCs reprogrammed from HD patient fibroblasts were genetically corrected for the *HTT* mutation using traditional homologous recombination via a targeting construct. Resulting isogenic iPSC pairs and differentiated isogenic NSC pairs were grown in tandem and plated as replicates for RNA isolation and RNA-seq analysis. There are eight biological replicates (BR) of each-corrected iPSCs, HD iPSCs, corrected NSCs, and HD NSCs: a total of 32 samples.

(B) Multi-dimensional scaling based on VSD-normalized expression levels of the top 5,000 most variable genes.

(C) Number of DE genes across comparisons at <0.01 false discover rate (FDR) threshold. Green bars represent the numbers of down-regulated genes and red upregulated for each comparison.

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