

PSEN1 Loss Sensitizes Developing Human Cortical Neurons To The Persistent Effects Of Methlymercury: A Proteomics-based Study

Ribhav Mishra¹✉ | Anke M. Tukker¹ | Hyunjin Kim¹ | Devender Arora²| V. P. Thirumalaikumar²| Aaron B. Bowman¹



¹ School of Health Sciences, Purdue University, West Lafayette, IN, USA | ² Bindley Bioscience Center, Purdue University, West Lafayette, IN, USA

INTRODUCTION

PSEN1 encodes the catalytic core in γ -secretase and is essential for amyloid precursor protein processing, mitochondrial function, and proteostasis, making it crucial in Alzheimer's disease (AD)biology. Developmental exposure to methylmercury (MeHg) is associated with long-term cognitive decline, yet its interaction with AD-relevant genetic risk factors remains poorly understood. Defining this gene-environment interaction is critical for understanding susceptibility of AD-vulnerable populations to early-life environmental toxicant exposure.

HYPOTHESIS

We hypothesized that loss of PSEN1 increases vulnerability to persistent molecular effects of developmental MeHg exposure, resulting in exaggerated proteomic and pathway-level disruptions compared with isogenic control.

EXPERIMENTAL DESIGN

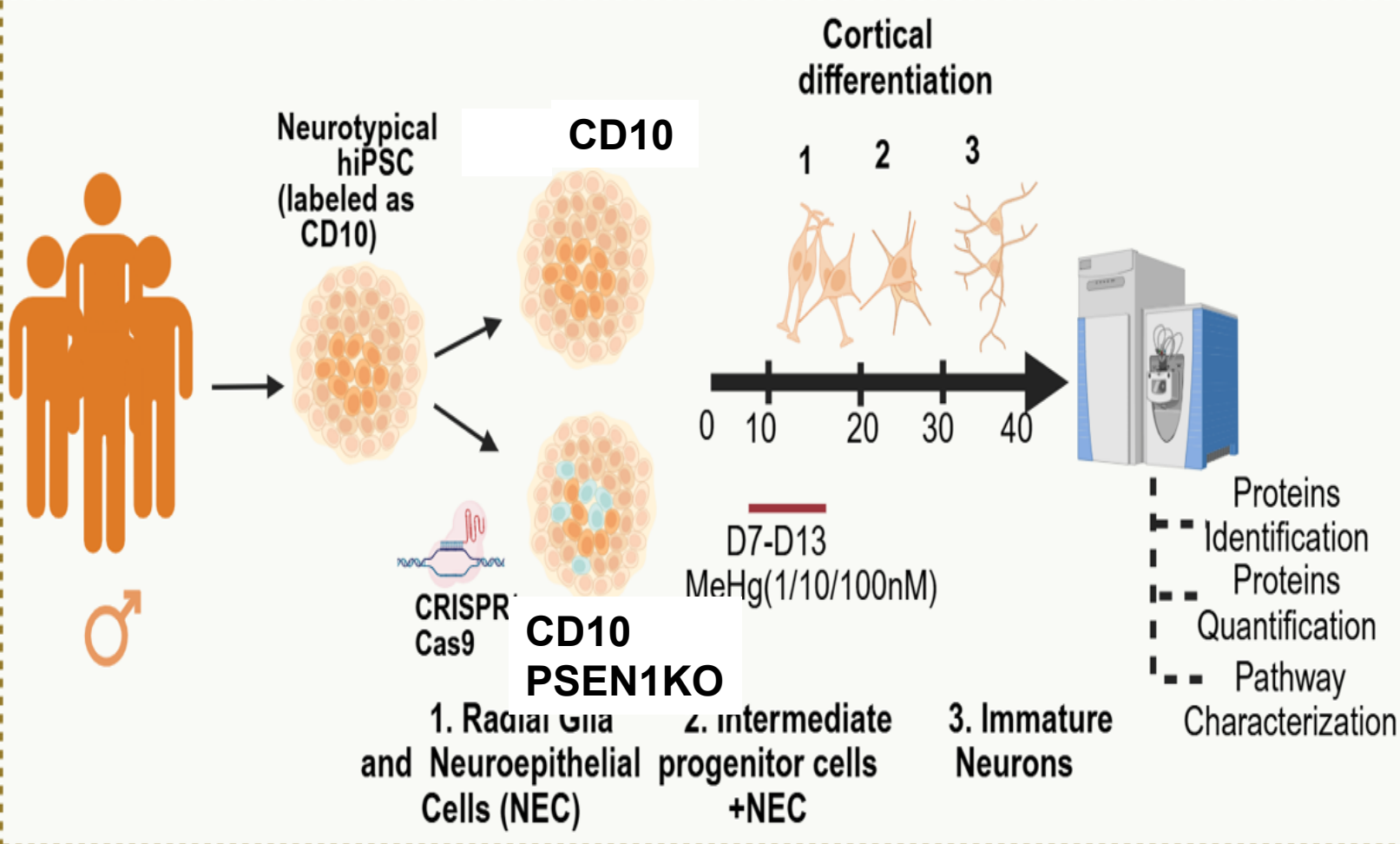


Fig 1. hiPSC cells (labeled as CD10) derived from healthy male subjects were modified using CRISPR-Cas9 approach to cause biallelic frameshift mutation in the PSEN1 gene resulting in knockout of PSEN1 gene. Afterwards, the isogenic control and the knockouts were differentiated (N=1 differentiation) to brain cortical lineage neurons and in the developmental phase (Days7-13) were exposed to different concentrations of MeHg.

Method: Generation of PSEN1 mutant

CD10 PSEN1 exon 4 :WT
5'CCATTATCTAATGGACGACC
CCAGGGTAACTCCCGGC-3'

↓ CRISPR/Cas9

CD10 PSEN1 Knock out (KO) Clone 51
5'CCATTATCTAATGGACGA^TCCCCAGGGT
AACTCCCGGC-3'
+1 bp T insertion
Allele 1: +T
Allele 2: +T
Homozygous Biallelic Frameshift Mutation

Fig 2. A homozygous biallelic insertion of T in PSEN1 gene in exon 4 by CRISPR-Cas9 resulted in frameshift mutation in the PSEN1 gene causing a knock-out of the PSEN1 gene and protein.

Proteomics Day 43 Data for CD10 and CD10PSEN1 KO hiPSC Lines with Developmental MeHg Exposure

Frameshift mutations in PSEN1 gene causes extensive proteomic remodeling

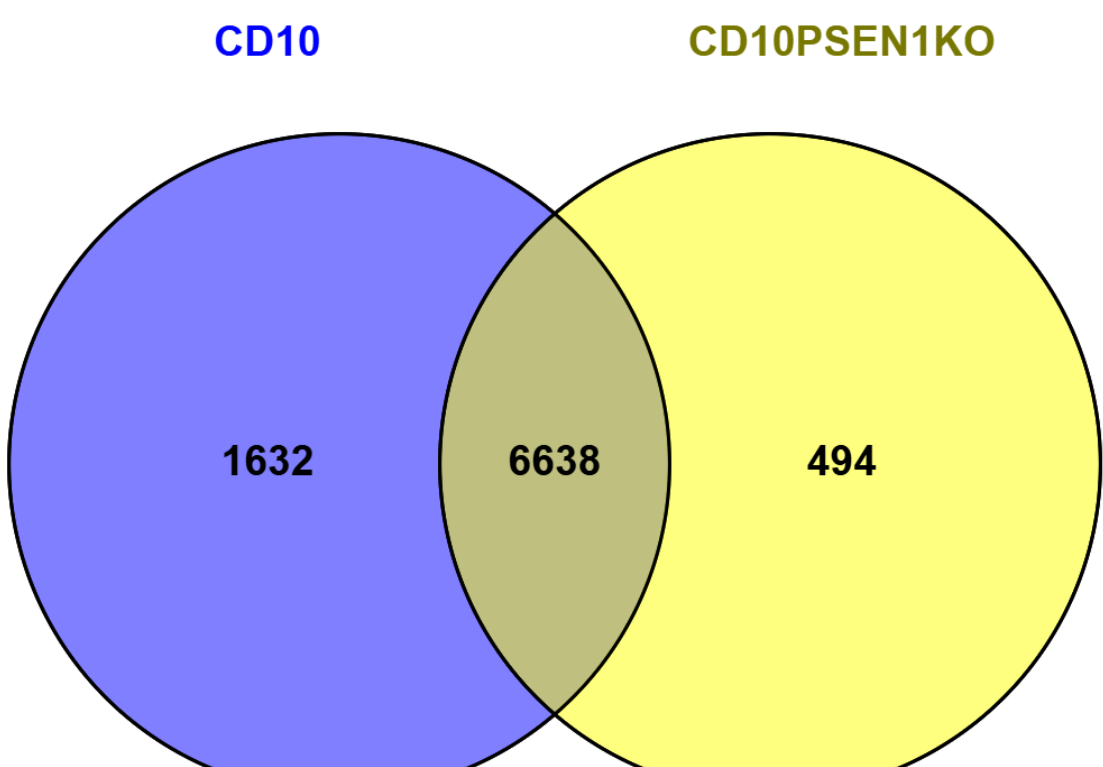


Fig 3.Venn diagram for all the proteins identified on Day43 of cortical differentiation (N=1 differentiation n=2 wells) of non-treated CD10 and CD10PSENKO lines by DIA based proteomic approach indicates that >6000 proteins are shared between PSEN1KO and its isogenic control and 494 new proteins(5.7% of total protein from isogenic control) are identified in CD10PSEN1KO line. Also, >1600 proteins are unique to isogenic control and is not present in the CD10PSEN1KO line

PSEN1KO cells exhibited a greater percentage of significantly changing proteins are upregulated as compared to its isogenic control across different MeHg exposure conditions

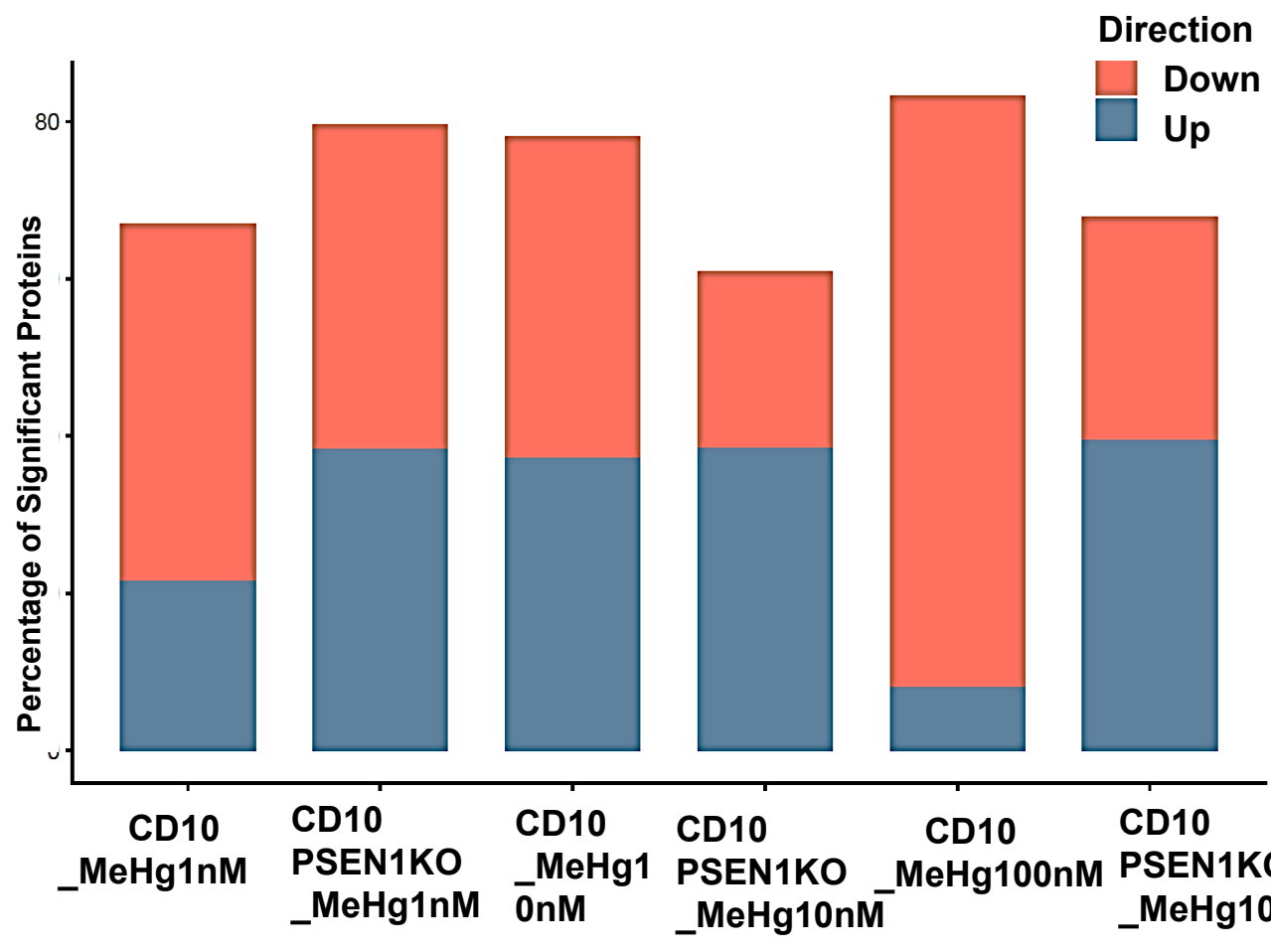


Fig5. Bar plot for the significant proteins by LIMMA based analysis (moderate t-test) identified on Day43 of cortical differentiation of CD10 and CD10PSEN1KO, indicates that a higher percentage of significantly changing proteins are upregulated in the group of CD10PSEN1KO cells

Increasing MeHg exposure leads to divergence in proteomic responses between PSEN1KO and control cells indicating a divergent proteomic changes at higher MeHg dose in the PSEN1KO cells

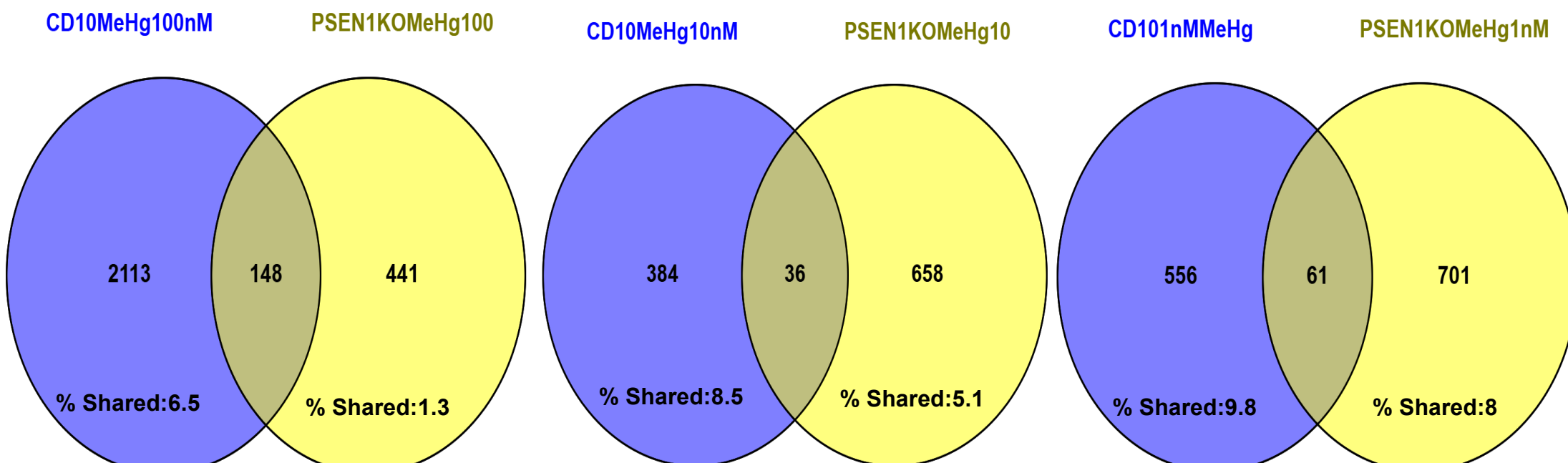


Fig 6. Venn diagram for significantly ($p \leq 0.05$) changing proteins as identified by proteomics for CD10 and CD10PSEN1KO lines (as prepared from Fig.4) are presented. The analysis indicates that as the dose of MeHg increases from 1nM to 100nM, there is decrease in the percentage of shared proteins between PSEN1KO and its isogenic control suggesting a divergent proteomics response at higher dose of MeHg 100nM. Statistical significance of %shared and non-shared protein was analyzed using Pearson's Chi-square test. CD10: $\chi^2(2)=8.78$, $p=0.012$; PSEN1KO: $\chi^2(2)=138.33$, $p<0.001$ indicating that PSEN1KO line is more significantly ($p<0.001$) and have a large effect $X^2=138.33$ by the lower MeHg concentration of 1nM.

PCA shows greater dose-dependent proteomic separation in PSEN1KO cells

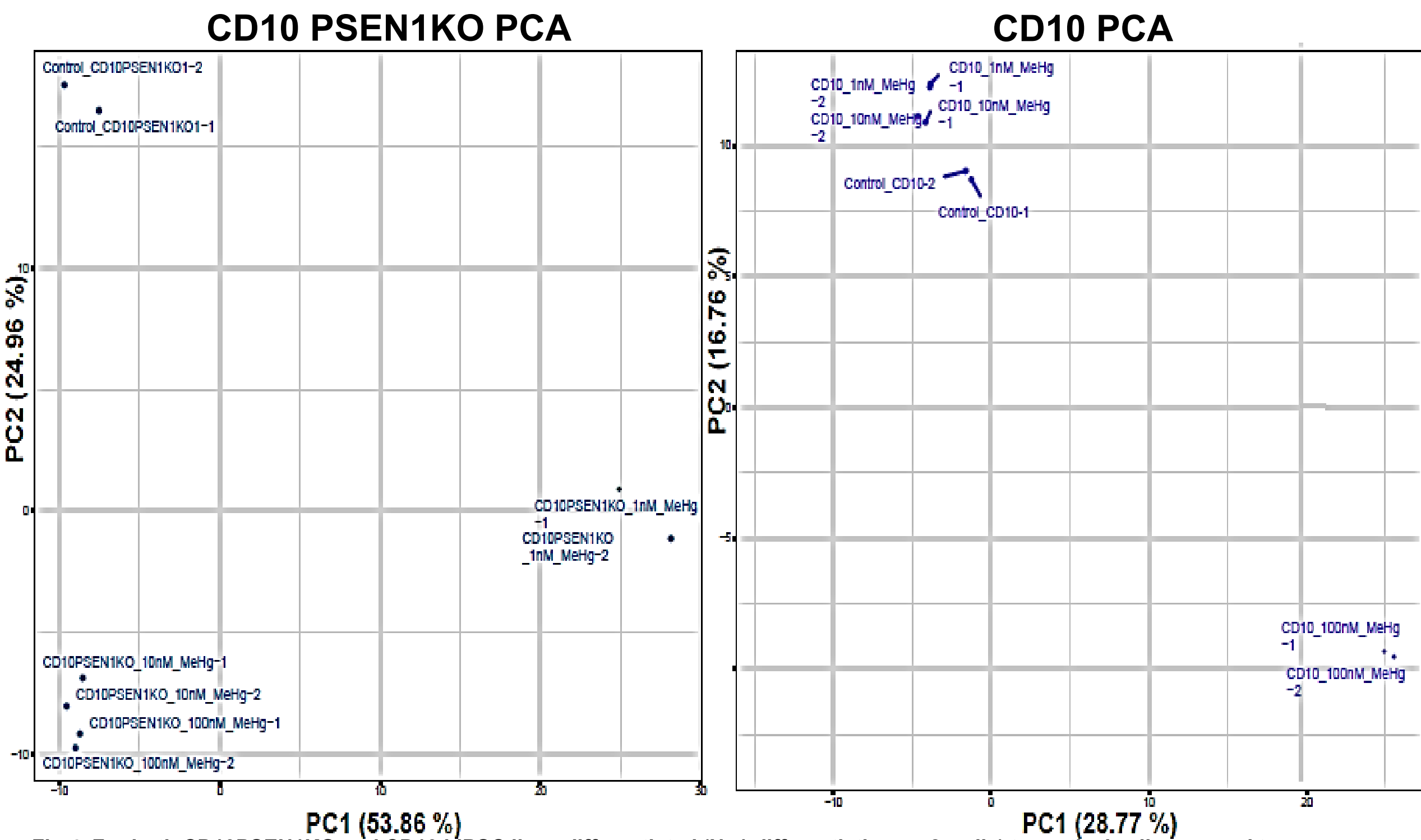


Fig 4. For both CD10PSEN1KO and CD10 hiPSC lines differentiated (N=1 differentiation, n=2 wells) to cortical cells exposed to different concentrations of MeHg (1/10/100nM) cell pellets were collected on D43 and were analysed by DIA proteomics. PCA analysis of proteomic changes in the CD10PSEN1KO line with MeHg developmental exposure indicates a dose-dependent response in the proteome compared to its isogenic control CD10. Additionally, the variation in proteome as indicated by PSEN1KOPC1 (53.8% for PSENKO) compared to CD10 control PC1(28.7%) indicates a higher variation after the PSEN1KO in the CD10 isogenic control.

Pathway enrichment analysis indicate that after PSEN1KO, cells become more sensitive to MeHg treatment and have a higher statistically significant changes in the pathways linked with metabolism (mitochondrial dysfunction, oxidative phosphorylation, resp. electron chain), cell-cycle (NOTCH1,G2-G1 M phase) and cell signaling (RHO-GTPase, DYRK1)

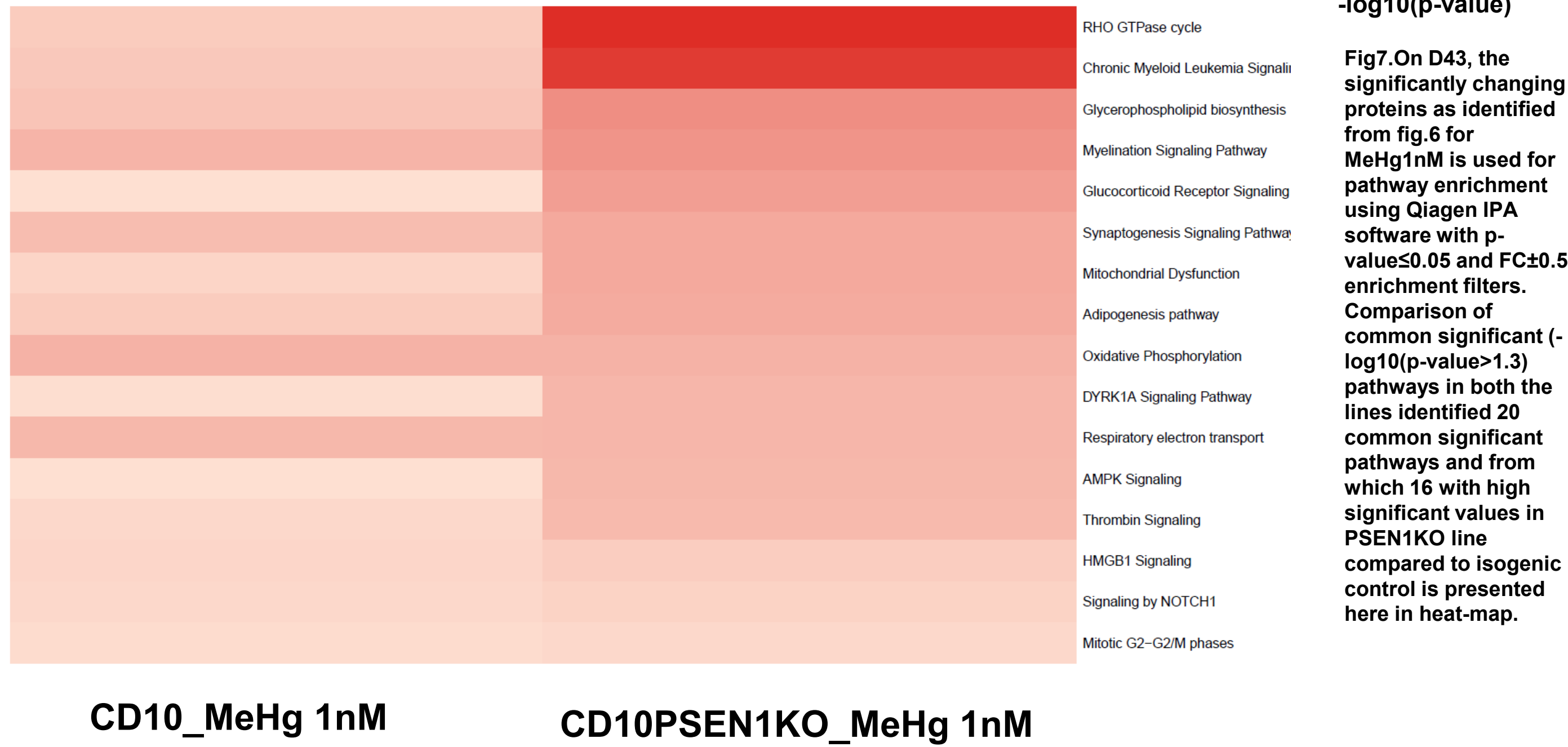


Fig7.On D43, the significantly changing proteins as identified from fig.6 for MeHg1nM is used for pathway enrichment using Qiagen IPA software with p -value ≤ 0.05 and $FC \geq \pm 0.5$ enrichment filters. Comparison of common significant ($-\log_{10}(p\text{-value}) > 1.3$) pathways in both the lines identified 20 common significant pathways and from which 16 with high significant values in PSEN1KO line compared to isogenic control is presented here in heat-map.