Dysregulation of the Wnt/β-catenin signaling pathway via Rnf146 upregulation in a VPA-induced mouse model of autism spectrum disorder

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ABSTRACT

Maternal exposure to valproic acid (VPA) during pregnancy is linked to autism spectrum disorder (ASD). This study used high-resolution mass spectrometry to analyze protein expression changes in the prefrontal cortex (PFC) of mice exposed to VPA in utero. The analysis revealed differential expression of proteins associated with ASD risk genes, particularly in the Wnt/β-catenin signaling pathway. Overexpression of Rnf146 in the PFC of adult mice resulted in regulatory abnormalities and social behavior defects in the Wnt/β-catenin pathway, along with increased excitatory synaptic metastasis in PFC neurons. We further examined the expression profile of Rnf146 in mouse forbrain. The findings suggest that Rnf146 disrupts the Wnt/β-catenin signaling pathway, contributing to ASD development following fetal exposure to VPA.

METHODS

Pregnant mice received E10 subcutaneous injections of VPA or saline, followed by social behavior tests at 6-7 weeks post-birth. PFC samples from VPA-exposed and control mice were collected for proteomic and western blot analyses, using high-resolution mass spectrometry and MaxQuant software. Enrichment analyses was performed in VPA-exposed mice, followed by functional mapping and protein-protein interaction network analysis. PFC samples from Rnf146-overexpressing mice underwent RNA sequencing, processed with Salmon software. Weighted gene coexpression network analysis (WGCNA) was applied for functional topology interpretation. Neuronal Rnf146 expression was achieved through an adeno-associated viral (AAV) vector, followed by targeted AAV injection via surgical procedures..

RESULTS

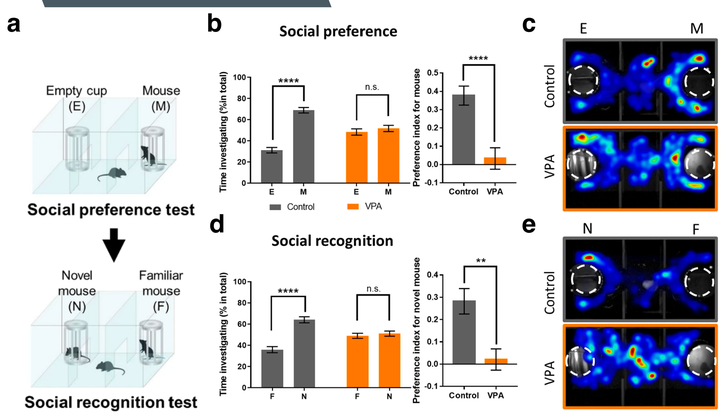

Figure 1. Social deficits in VPA-exposed mice. a. Schematic diagram of the three chamber social behavior test. **b.** Social preference test and preference index between VPA-exposed mice (n = 19) and control mice (n=20). **c.** Representative heatmap images of the social preference test of control and VPA-exposed mice. **d.** Social recognition test result and preference index between VPA **e.** Representative heatmap images of the social recognition test of control and VPA-exposed mice.

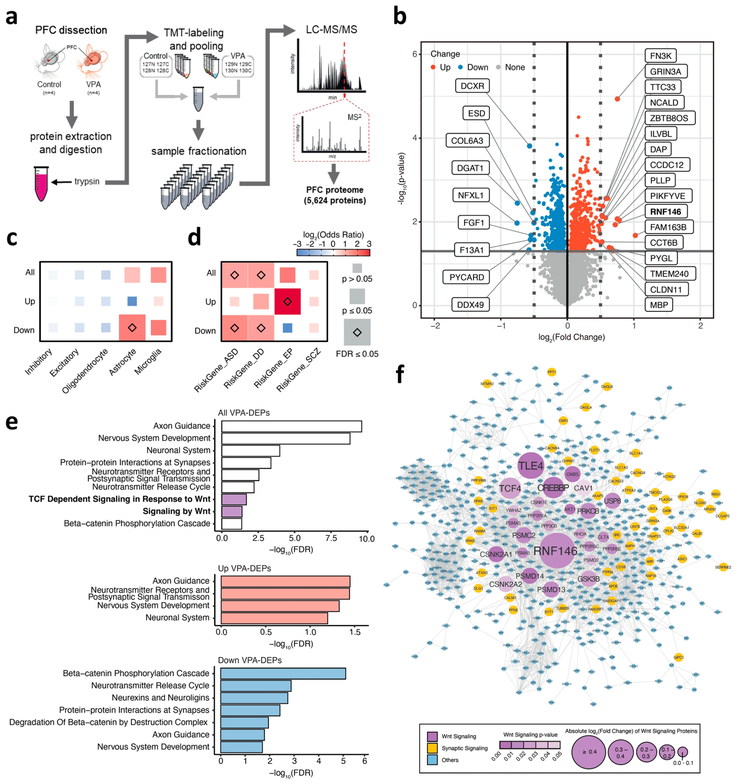

Figure 2. VPA-exposed mice show upregulation of the Wnt signaling pathway. a. The overall design of the proteomic experiment. **b.** Volcano plot showing DEPs between VPA-exposed and control mice. **c.** Enrichment analysis of VPA-DEPs with mouse brain celltype markers. **d.** Enrichment analysis of VPA-DEPs with disorder risk genes. Autism spectrum disorder (ASD), developmental disorder (DD), epilepsy (EP), and schizophrenia (SCZ). **e.** Functional annotations for VPA-DEPs showing significantly enriched biological pathways (FDR ≤ 0.05). **f.** Protein-protein interaction network of VPA-DEPs related to Wnt signaling and neuronal development.

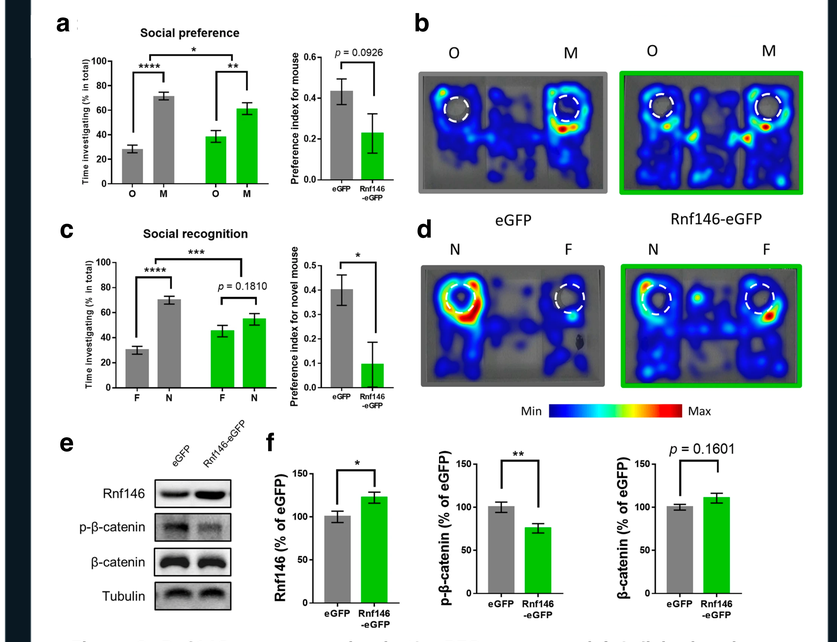

Figure 3. Rnf146 overexpression in the PFC causes social deficits in mice. a. Social preference test and preference index between Rnf146-overexpressing mice compared to eGFP-overexpressing control mice. **b.** Representative heatmap images of the social preference test. **c.** Social recognition test result and preference index between Rnf146- and eGFP-over expressing mice. **d.** Representative heatmap images of the social recognition test. **e.** Western blot analysis result. **f.** Changes of Rnf146, p-β-catenin, and β-catenin levels in Rnf146 overexpression.

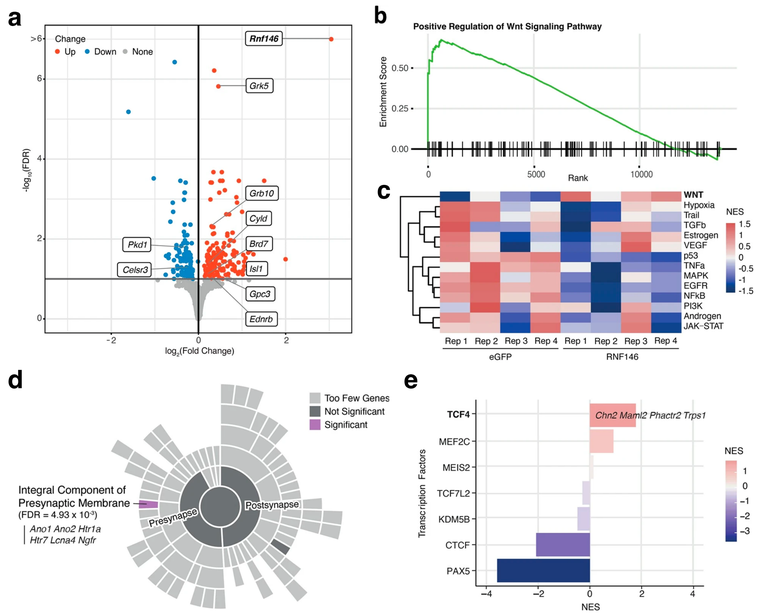

Figure 4. The Wnt signaling pathway is promted by Rnf146 overexpression a. Volcano plot showing DEGs between Rnf146- and eGFP-overexpressing mice. **b.** Enrichment plot depicting the elavated positive regulation of Wnt signaling pathway in Rnf146-overexpression. **c.** Heatmap of the pathway activity inferred by PROGENy. **d.** Enrichment of upregulated Rnf146-DEGs with synaptic locations. **e.** Enrichment with inferred transcription factor activities associated with ASD.

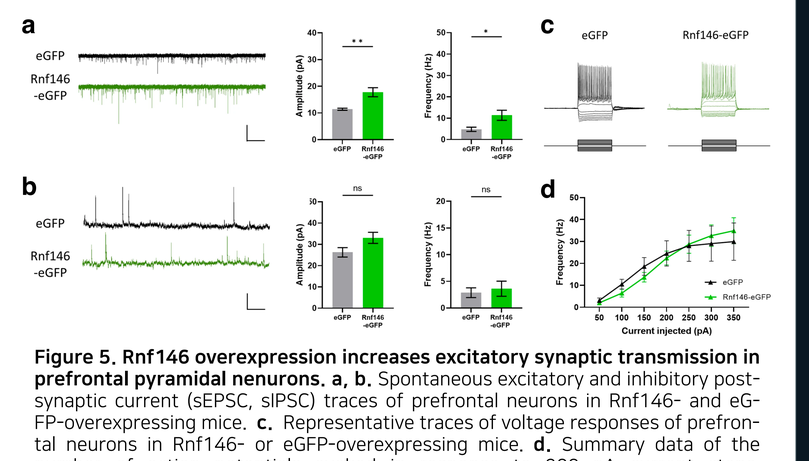

Figure 5. Rnf146 overexpression increases excitatory synaptic transmission in prefrontal pyramidal neurons. a, b. Spontaneous excitatory and inhibitory postsynaptic current (sEPSC, sIPSC) traces of prefrontal neurons in Rnf146- and eGFP-overexpressing mice. **c.** Representative traces of voltage responses of prefrontal neurons in Rnf146- or eGFP-overexpressing mice. **d.** Summary data of the number of action potentials evoked in response to 300 pA current steps.

DISCUSSIONS

Prenatal exposure to valproic acid (VPA) induces ASD-like social deficits, linked to increased Rnf146 expression activating the Wnt/β-catenin pathway. Rnf146 overexpression leads to impaired social behavior through heightened excitatory synaptic transmission. Dysregulation of the Wnt signaling is identified as a contributor to ASD etiology, impacting development and synaptic functions. These findings offer new insights into the molecular mechanism of VPA-associated ASD, suggesting the Rnf146-Wnt/β-catenin pathway as a potential target to alleviate social deficits in ASD.

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