Title: ALTERNATIVE SPLICING OF A VOLTAGE-GATED CALCIUM CHANNEL IN THE CEREBELLUM AND SCHIZOPHRENIA

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The voltage-gated calcium channel, $Ca_V2.1$, is the most abundant presynaptic calcium channel and is highly expressed in the soma and dendrites of Purkinje cells of the cerebellum. There are two mutually exclusive splice variants in exon 37 of cerebellar CaV2.1, EFa and EFb, with EFa being the predominant form. Whilst $Ca_V2.1$ channelopathies usually result in neurological disorders such as hemiplegic migraine 1 (FHM-1), Episodic Ataxia 2 (EA2), Spinocerebellar ataxia type 6 (SCA6) and epilepsy, EA-2 and SCA6 patients also exhibit symptoms of schizophrenia (SCZ). A recent study of post-mortem whole brain tissue from patients with ASD, SCZ, bipolar disorder, as well as healthy controls, revealed alterations in alternative splicing of the gene encoding $Ca_V2.1$. Moreover, neuroimaging studies and clinical interventions have also implicated the cerebellum in SCZ pathology. These diverse lines of evidence converge upon a novel hypothesis: alternative splicing of $Ca_V2.1$ in the brain, particularly the cerebellum, could underlie the molecular pathology of SCZ.

To examine the contributions of $Ca_V2.1$ alternative splicing to cerebellar dysfunction, we generated a conditional knockout mouse model with selective deletion of EFa (EFa-cKO) in Purkinje cells (PC), the sole output neurons of the cerebellar cortex. EFa-cKO mice are ataxic from postnatal day 12 onwards and immunohistochemical analysis of the cerebellum revealed a selective degeneration of PCs in aldolase C (-) compartments (zebrin-negative bands) beginning at postnatal day 28.

Because $Ca_V2.1$ channels play an essential role in triggering neurotransmitter release, defects in splicing of $Ca_V2.1$ in PCs could affect inhibitory synaptic transmission between PCs and neurons of the deep cerebellar nuclei (DCN), the major source of output from the cerebellum to the rest of the brain. To test this possibility, we recorded inhibitory postsynaptic currents (IPSCs) from DCN neurons while electrically stimulating axons of presynaptic PCs in cerebellar slices. Evoked IPSC amplitudes in EFa-cKO mice were significantly smaller than those in wild-type mice (301 ± 60 pA vs. 1170 ± 274 pA). Activity-dependent plasticity was also altered at this synapse: EFa-cKO mice showed more prominent synaptic facilitation compared to wild-type mice. These alterations in synaptic strength and plasticity at PC-DCN synapses will affect cerebellar information processing, leading to cognitive and behavioural dysfunction.

To examine this possibility, we performed behavioural analyses on EFa-cKO mice. In the three-chamber social interaction test, EFa-cKO mice spent significantly less time interacting with the cage containing the stranger mouse, indicating a deficit in social behaviour. EFa-cKO mice had significantly less spontaneous alternation in the Y-maze, as compared to wild-type mice, suggesting reduced working memory and/or impaired spatial memory in EFa-cKO mice. In the marble burying assay, EFa-cKO mice buried more marbles than the wild-type mice, indicating an increase in stereotypical behaviour or anxiety. These behavioural changes parallels observations in SCZ patients.

Our results suggest that defects in $Ca_V2.1$ splicing in the cerebellum could contribute to SCZ.