UNIVERSITÀ DEGLI STUDI DI MILANO

D-MEM PhD-UNIMI | Doctorate program Milan EXPERIMENTAL MEDICINE

PROJECT PROPOSAL Francolini- XXXVIII cycle

PROJECT TITLE	Analysis of GABAergic circuitry in Pcdh19 mouse model of Developmental and epileptic encephalopathy 9 (DEE9)
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PROJECT Description	Mutations in the X-chromosome gene *PCDH19* cause Developmental and Epileptic Encephalopathy-9 (DEE9). The mutations are associated to epilepsy, autistic spectrum disorders, and cognitive impairment. DEE9 is characterized by a peculiar gender preference, as it affects only females. Rare DEE9 males are somatic mosaic. We demonstrated that in vivo downregulation of Pcdh19 in the hippocampus affects migration and morphological maturation of neurons and GABA circuitry. We also found that brains of conditional *Pcdh19* KO mice are characterized by a global reduction of network activity and an increased neuronal synchronization. Furthermore, in freely behaving mice *Pcdh19* mosaic expression compromises the excitation/inhibition (E/I) balance leading to the formation of aberrant functional connections within the limbic system. Based on these data, we believe that defects in Protocadherin19 expression in GABAergic neurons might be one of the mechanisms altering GABAergic signaling and E/I balance in DEE9. Hence, we plan to examine the consequences of Pcdh19 deletion in inhibitory neurons at the molecular, cellular and functional level in mice thus, possibly, to identify new targets and to develop novel therapies for patients with DEE9. To do this, we will use the *Pcdh19 fl /fl* mouse in which the expression of PCDH19 is silenced in inhibitory interneurons through intracerebroventricular injection at P0 of adeno-associated viral vectors expressing Cre recombinase and fluorescent protein (FP) under the control of the GABAergic interneuron-specific Dlx5/6 promoter (AAV.PHP.B-mDlxp-FP-2A-iCre) designed as *cPcdh19 mDLxCre* mice. As control we will use AAV.PHP.B-mDlxp-FP-2A-ideltaCre. We will focus on the hippocampus and the pre-frontal cortex and to exclude or alternatively to identify gender related-phenotypes, we will perform our experiments in separate groups of mice of both sexes. We will investigate if the absence of PCDH19 in inhibitory interneurons alters the formation and maturation of synapses in the hippocampus and pre-frontal cortex. These results will be associated with the characterization of the electrophysiological properties and network activity in the hippocampus and pre-frontal cortex of *cPcdh19 mDLxCre* mice; experiments that will be carried on by our collaborators of the CNR - Institute of Neuroscience. Our goal is to deepen the knowledge of the pathological mechanisms of DEE9 and provide the first insight into GABAergic signaling involvement. PCDH19 has become the second most important gene in epilepsy after SCN1A but no effective therapies exist for DEE9. To overcome this, there is the need to expand our knowledge of PCDH19 biological role and the mechanisms underlying PCDH19-related pathology of interneuronal circuits.