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MEETING ABSTRACT

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The pathogenic, autism-linked *de novo* variant A749G in $Ca_V1.3$ Ca^{2+} channels affects neuronal morphology *in vitro* and *in vivo*

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Background: Due to activation at subthreshold potentials, in brain, voltage-gated L-type $Ca_V1.3$ calcium channels (*CACNA1D*) contribute to pacemaking activity in neurons and regulate synapse maturation and dendritic refinement. Functional studies from our group revealed that *de novo* missense mutations in the $Ca_V1.3$ α1 subunit, such as variants A749G and S652L, identified in patients with neurodevelopmental disorder, induce prominent gain-of-function gating changes [1]. However, whether these pathogenic mutations influence dendritic and synaptic structure remains unclear.

Methods: Here, we investigated the effect of the autism-causing $Ca_V1.3$ variant A749G on neuronal morphology in transfected hippocampal cultures and Golgi-Cox-stained CA1 hippocampal neurons from heterozygous A749G knock-in mice ($Ca_V1.3^{A749G}$).

Results: In cultured neurons the A749G mutation introduced into the either HA-tagged or untagged $Ca_V1.3$ long splice variant induced a significant increase in dendritic spine length, fiber length and spine area. Cumulative frequency distribution of spine shape factor was significantly shifted to lower values compared to wild-type (WT) indicating an elongation of the entire spine population. Consequently, the percentage of thin and filopodia-like spines was increased in neurons transfected with mutant channels. Our anti-HA live-cell staining did not reveal major differences between WT and mutant channels surface expression. This indicates that the mutation-induced spine elongation does not result from altered channel membrane targeting. In Golgi-Cox-stained brains of 3- to 3.5-months-old heterozygous $Ca_V1.3^{A749G}$ mice we observed a significant increase of branching in proximal dendritic regions of CA1 hippocampal neurons compared to WT. Moreover, dendritic spine analysis revealed an increase in the proportion and density of stubby spines and a decrease in the proportion and density of thin spines in basal and apical dendrites. Additionally, neuronal soma size was significantly reduced in CA1 hippocampal neurons from $Ca_V1.3^{A749G}$ mice.

Discussion: In this study, we demonstrated that the pathogenic, autism-linked mutation A749G in $Ca_V1.3$ affects neuronal morphology both *in vitro* and *in vivo*. Differences in mutation-induced effects on dendritic spines observed between hippocampal cultures and mouse brains can be explained by: (i) differences in $Ca_V1.3$ expression levels leading to distinct contributions of $Ca_V1.3$ to intra-spine Ca^{2+} levels and therefore to spine stability; and (ii) differences in the levels of neuronal development represented by two systems. Additionally, a more stable neuronal network is developed in intact mouse brain compared to embryonic neurons differentiated *in vitro*. Our data strongly suggest that $Ca_V1.3$ is required for normal neuronal morphology in hippocampal neurons. The changes induced by the pathogenic A749G $Ca_V1.3$ variant may contribute to the neurodevelopmental pathology in affected patients.

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Keywords: voltage-gated calcium channels – $Ca_V1.3$ channels – gain-of-function mutations – dendritic spine morphology – dendritic arborization – neurodevelopmental disorders

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