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INTRINSIC ACTIVITY

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MEETING ABSTRACT

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The human A749G *CACNA1D* ($Ca_V1.3$) variant alters channel gating and causes a phenotype in mice similar to the human neurodevelopmental disorder

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Background: *De novo* missense variants of *CACNA1D*, encoding the pore-forming α1 subunit of $Ca_V1.3$ L-type Ca^{2+} channels (LTCCs), are associated with treatment-resistant hypertension and a neurodevelopmental syndrome that can also manifest with endocrine features (hyperaldosteronism, hyperinsulinemic hypoglycemia). Patch-clamp recordings revealed mutation-induced alterations in channel gating that are predicted to promote $Ca_V1.3$ channel activity at subthreshold potentials. However, to date definite proof of their disease-causing nature is still missing.

Methods: To study the pathophysiological consequences of such gating-modifying *CACNA1D* variants, we have introduced the A749G variant found in a patient with autism spectrum disorder and intellectual disability into C57BL/6N mice ($Ca_V1.3^{AG}$ mouse line). We have characterized $Ca_V1.3^{AG}$ mice using behavioural, neuroanatomical and electrophysiological methods, and conducted an *in vivo* pharmacological rescue experiment using the LTCC inhibitor isradipine (extended-release formulation).

Results: $Ca_V1.3^{AG}$ mutant mice are viable, reproduce and appear overall healthy except for a delayed gain of body weight (more pronounced in homozygous mutants). Patch-clamp recordings in cultured mouse chromaffin cells from heterozygous mutants confirmed altered native LTCC Ca^{2+} currents resembling gating changes observed upon heterologous expression of A749G-containing $Ca_V1.3$ channels. While plasma aldosterone levels were elevated only in adult female mutants, blood glucose levels at baseline and after an i.p. glucose challenge were significantly decreased in adult homozygous males only, indicating a sexual dimorphism. Mutants of both sexes displayed increased locomotion induced by handling and/or a novel environment in a gene-dose-dependent manner. Further behavioral analysis of adult male mice identified an anxiety-like behavior in the light-dark box, absent marble-burying behavior (homozygous mice only), altered grooming and rearing patterns and a social deficit (3-chamber test). Gross neuroanatomy was unaltered, whereas dendritic spine morphology of CA1 pyramidal neurons in the dorsal hippocampus in Golgi-Cox-stained brain sections of adult $Ca_V1.3^{AG}$ mice was altered (see poster by Nikonishyna *et al.* [1] for details). Electrophysiological recordings in acute brain slices revealed increased cellular excitability in striatal medium spiny neurons and medial dopaminergic substantia nigra neurons projecting to the dorsomedial striatum in heterozygous mutants. Finally, oral pretreatment over 2 days with 0.3 or 1–2 mg/day isradipine (doses based on a separate pharmacokinetic study) resulted in therapeutically relevant plasma levels but did not rescue the hyperlocomotive phenotype in female or male mice, respectively.

Discussion: Here, we provide the first direct proof for the pathogenicity of a gating-modifying *CACNA1D* missense variant and demonstrate that our construct-valid disease mouse model can be used to study disease-underlying mechanisms as well as therapeutic interventions.

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Reference:

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