

A De Novo CACNA1S Mutation in Cattle Reveals Insights into Congenital Myopathy-18 Pathophysiology

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INTRODUCTION

Congenital myopathies are skeletal muscle disorders present at birth, with onset ranging from early to late infancy or even adulthood. Congenital myopathy-18 (CMYO18) is caused by homozygous or heterozygous mutations in CACNA1S, which encodes the skeletal muscle voltage-gated calcium channel (CaV1.1). CMYO18 patients present with hypotonia, progressive muscle weakness, swallowing difficulties, respiratory insufficiency, ptosis, a high-arched palate, and ophthalmoplegia. While only a few cases have been reported, the underlying mechanism remains unclear.

Here, we characterize a de novo CACNA1S missense mutation in the pore-forming segment of CaV1.1. Identified in Holstein cattle, this mutation led to a CMYO18-like phenotype in newborn and young animals, yet a cohort of adult homozygous animals showed no apparent symptoms. Affected animals displayed muscle fiber size variability, increased cellularity, and sporadic internal nuclei, but no core-like disorganization. Channel expression was reduced by 50–70% in the locomotor and respiratory muscles of young animals.

Overexpression of the mutated channel in adult mouse muscle did not affect excitation-contraction coupling, yet calcium current loss-of-function was evident. Biophysical analysis in dysgenic myotubes revealed reduced conductance and defects in channel trafficking, impairing excitation-contraction coupling.

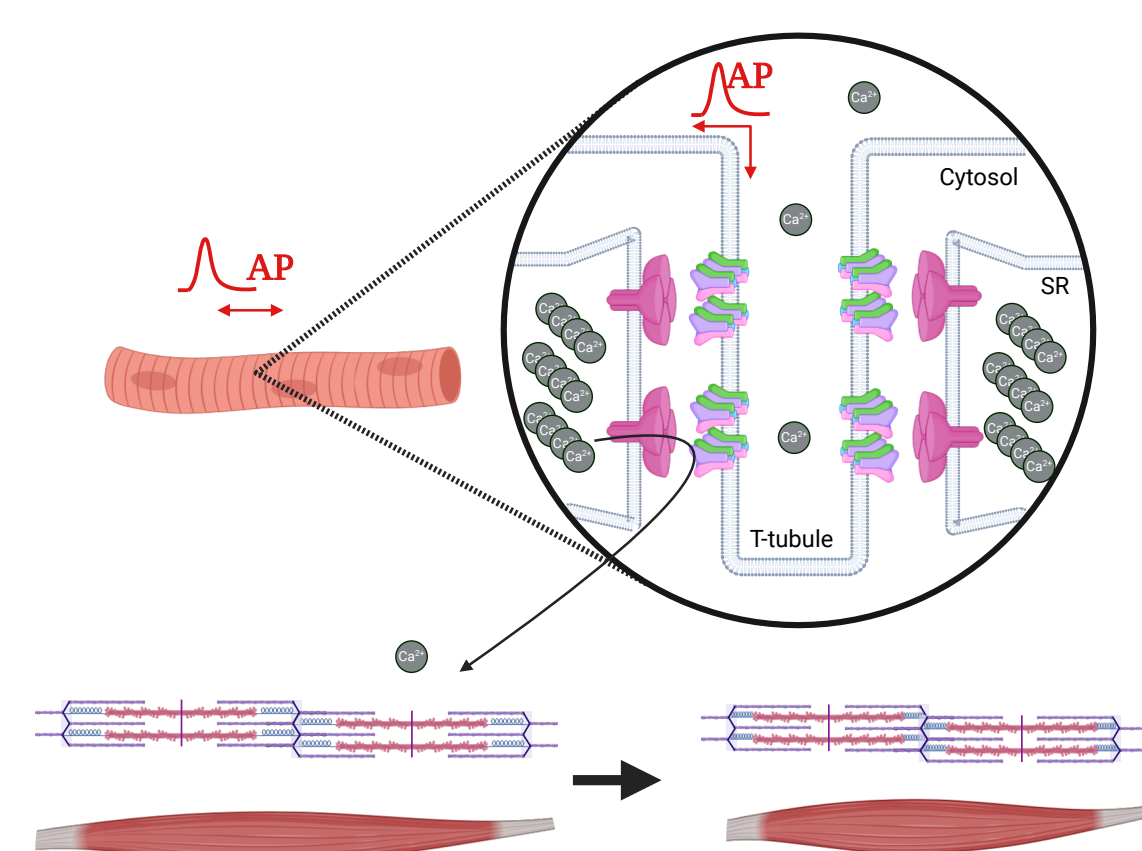
As in other mammals, the embryonic CaV1.1 variant (CaV1.1Δ29) is expressed in cattle. Ongoing studies aim to determine whether CaV1.1Δ29 harboring this mutation explains early disease onset while the absence of symptoms in adulthood. Our findings suggest that CaV1.1 trafficking defects underlie CMYO18 and that promoting channel expression, even with missense mutations in pore segments, may offer a potential therapeutic strategy to restore excitation-contraction coupling.

BACKGROUND

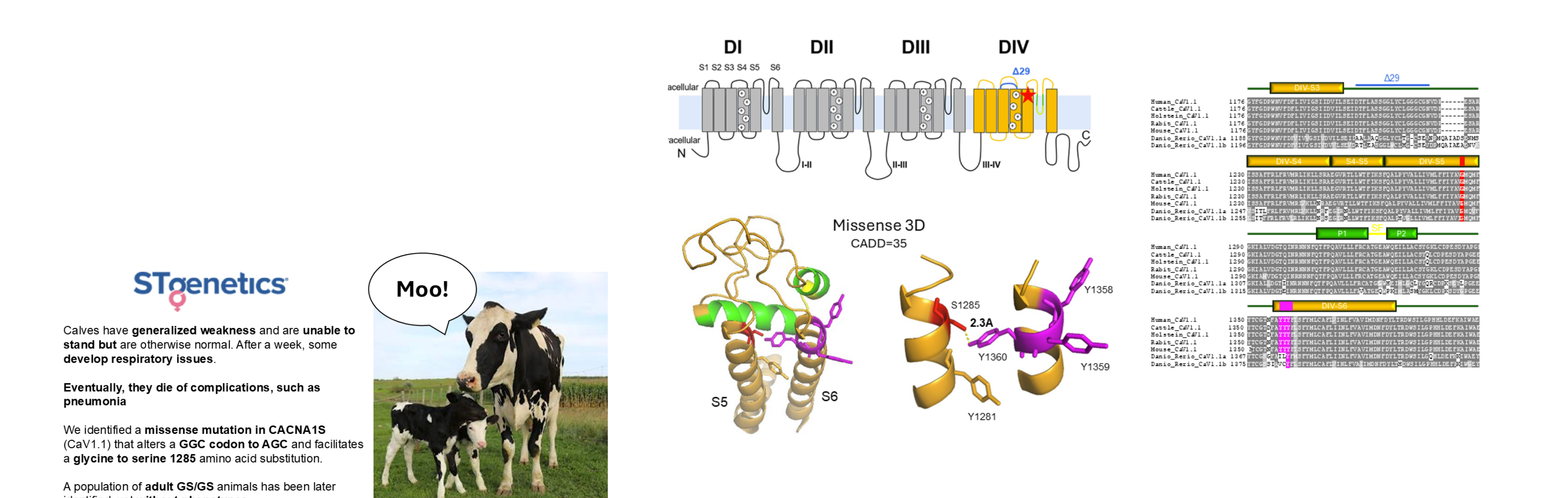
The skeletal muscle L-type Ca²⁺ channel (CaV1.1) has two primary functions: (1) the voltage-sensor for Ca²⁺ release from the sarcoplasmic reticulum (SR) via the type 1 ryanodine receptor (RyR1), and (2) L-type Ca²⁺ channel.

CaV1.1 is expressed exclusively in skeletal muscle at triad junctions formed by the plasma membrane of the transverse-tubule network (T-tubules) and the terminal cisternae of the SR. CaV1.1 channels are clustered in groups of four (tetrad) within the plasma membrane of triad junctions; and each tetrad is juxtaposed to one leaf every other quatrefoil RyR1 in the SR membrane. This unique ultrastructure is a prerequisite for the intermolecular communication between CaV1.1 and RyR1 that supports excitation-contraction coupling (ECC) in skeletal muscle.

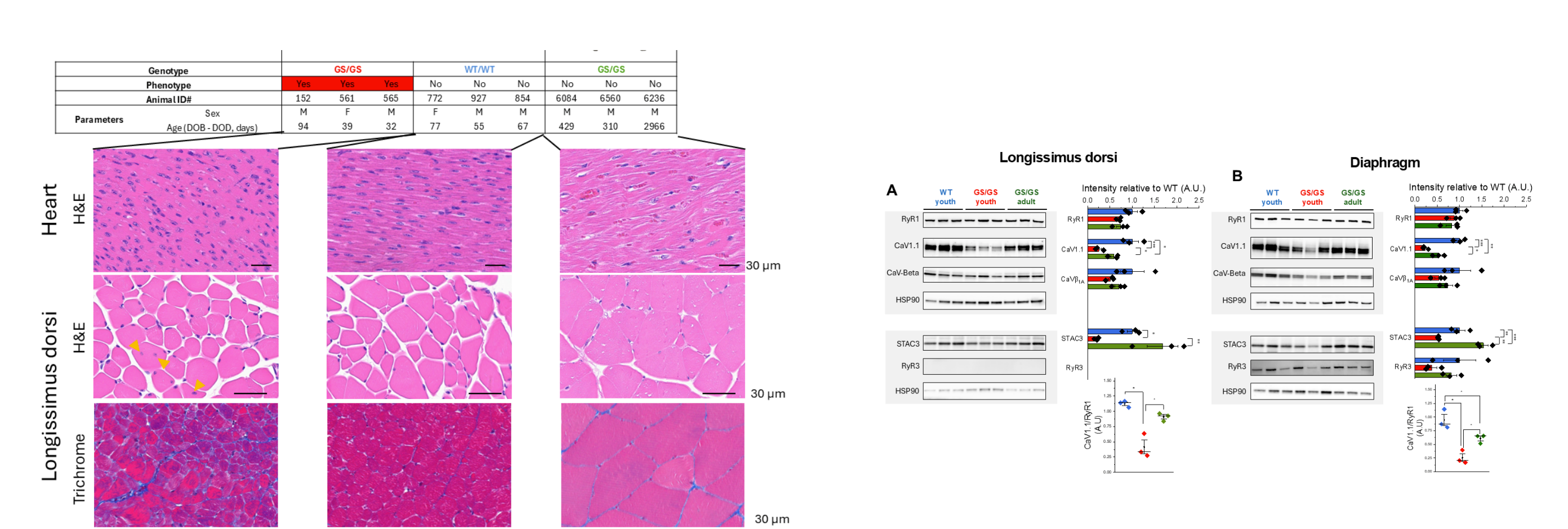
In response to the muscle action potential, CaV1.1 undergoes molecular rearrangement which activates coupled RyR1 channels. RyR1-rapid Ca²⁺ release from the SR into the cytosol lead to Ca²⁺ binding to troponin C and activation of actin-myosin interactions for muscle contraction. Membrane depolarization also activates Ca²⁺ influx through CaV1.1, yet Ca²⁺ flux is disposable ECC in differentiated mature skeletal muscle.



RESULTS

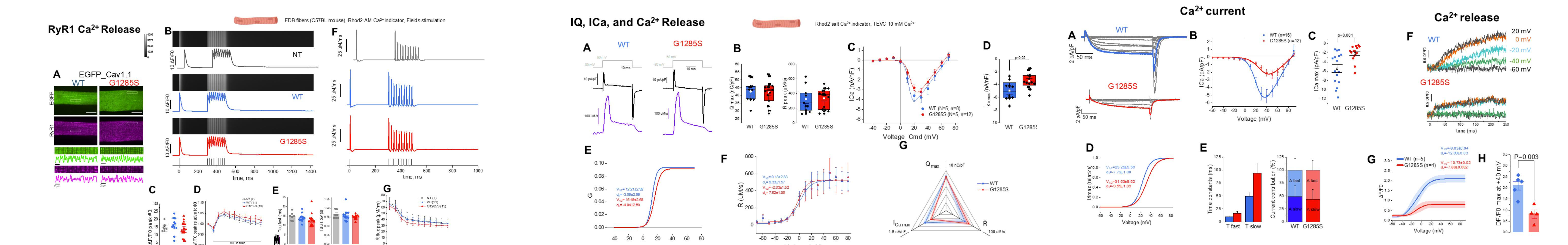


CaV1.1 G1285S mutation is near the extracellular end of S5_{iv}. The substitution of the glycine (G) with a serine (S) residue does not affect the overall charges, but serine side chain could cause steric modifications. The overall CaV1.1 sequences is highly conserved within mammals. The serine residue could participate in possible clashes with the neighboring S5_{iv} Y1281 or S6_{iv} Y1358, Y1359, and Y1340. Serine 1285 side chain and Tyrosine 1340 are distant of 2.3 Å, compatible with hydrogen bond formation. The missense is predicted pathogenic (CADD 35).



Tissues from 3 homozygous affected young animals (GS/GS), 3 controls animals (WT/WT) and 3 homozygous non affected adult animals (GS/GS adult) have been used for histology. H&N stains of the heart did not reveal any identifiable defects. H&N stains show an increase in cellularity, increased roundness of the fibers and marginal central nuclei in GS/GS but not GS/GS adult. Trichrome stains show an increase of collagen deposits GS/GS but not GS/GS adult. These results are coherent with the muscle weakness phenotype in youth GS/GS but not adult GS/GS animal, suggesting a transient or developmental only defect.

Tissues from the same 3 homozygous affected young animals have been used for Western blot. No changes have been observed in the expression of RyR1, yet a decrease >70% has been observed in GS/GS in diaphragm and biceps femoris. CaV1.1 subunit STAC3 is also significantly decreased and CaV1.1 subunit CaV_Beta1A is reduced, yet more marginally.

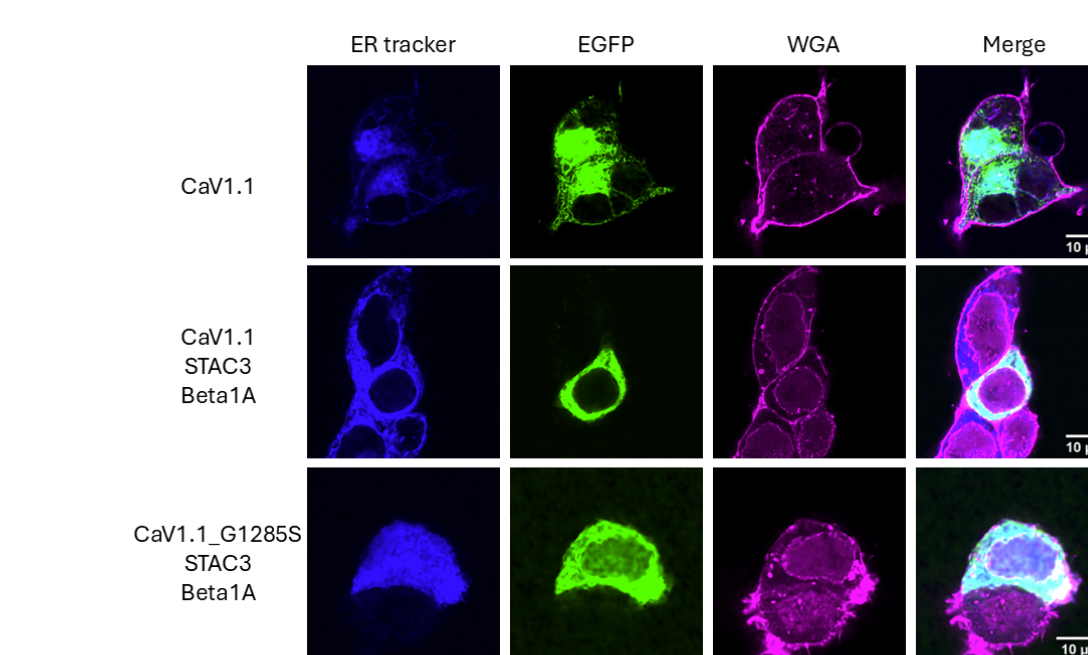


Electroporation of EGFP_CaV1.1_G1285S in mouse FDB muscle show expression at the t-tubule membrane (A). Upon field stimulation, G1285S transfected muscle supports robust action potentials induced Ca²⁺ transient that does not differ from WT or non-transfected muscle (B-E). Utilization of mathematical Ca²⁺ removal model shows no difference in Ca²⁺ released from the SR by RyR1 (F-G).

FDB muscles electroporated with EGFP_CaV1.1_G1285S or _WT show no difference in Ca²⁺ release from RyR1 nor gating Charge (IQ) from CaV1.1 under voltage clamp (A-B). L-Type Ca²⁺ current from CaV1.1 is slightly but significantly reduced (C-D), yet no change in voltage dependence is observed (E). No change is observed in RyR1 Ca²⁺ release voltage dependence (F). Overall, this suggests that G1285S traffic properly at the membrane (IQ) in adult muscle and support proper RyR SR Ca²⁺ release; yet G1285S may impair CaV1.1 conductance in adult muscle (G).

GLTs cells are immortal muscles cell line expressing RyR1 but KO for CaV1.1. Transfection of CaV1.1 WT and G1285S in GLTs allow restoration of Ca²⁺ current (A). Yet, G1285S current amplitude is reduced (B-C), a small shift in current activation is observed (D), and current activation is slower (E). RyR1 Ca²⁺ release is significantly decreased in G1285S in comparison to WT, without any effect in voltage dependence. Overall, this suggests that in developmental muscle cells, G1285S is not trafficked properly at the membrane, that the channel expressed conduct less Ca²⁺, and most RyR1 channels remain uncoupled to CaV1.1.

ONGOING



When CaV1.1 alone is transfected in tsA cells, it is not trafficked to the membrane, and it is retained in the ER (Top). Co-expression of CaV1.1 + STAC3 + Beta1A allows trafficking of the channel at the membrane (Middle). In tsA, CaV1.1_G1285S + STAC3 + CaV_beta1A is expressed a little at the membrane and is mostly retained in the ER (Bottom). This indicate that G1285S impair channel folding and trafficking at the membrane.

CONCLUSION

CaV1.1 is assembled in the endoplasmic reticulum (ER), processed in the Golgi apparatus, and then trafficked to the plasma membrane. At the membrane, it organizes into tetrads positioned opposite RyR1. Upon activation, CaV1.1 facilitates the release of calcium (Ca²⁺) from the sarcoplasmic reticulum (SR) into the cytosol, triggering muscle contraction. This Ca²⁺ also binds to transcription factors that translocate to the nucleus contributing to overall Ca²⁺ signaling. In developing or respiratory muscles, CaV1.1 can additionally activate RyR3 via a calcium-induced calcium release (CICR) mechanism, further contributing to overall Ca²⁺ signaling. In the case of CMYO18, CaV1.1 may accumulate in the ER, reducing its availability at the membrane and thereby impairing Ca²⁺ release. In the G1285S variant, if the animal survives the early postnatal period, sufficient expression of CaV1.1 can support normal development into adulthood. Therapeutic strategies aimed at improving CaV1.1 folding or transiently enhancing RyR1-mediated Ca²⁺ release could potentially alleviate the phenotype associated with CMYO18.

