

Mutation associated with an autosomal dominant cone-rod dystrophy *CORD7* modifies RIM1-mediated modulation of voltage-dependent Ca²⁺ channels

Takafumi Miki¹, Shigeki Kiyonaka¹, Yoshitsugu Uriu¹, Michel De Waard²,
Minoru Wakamori^{1,3}, Kevin P. Campbell⁴ & Yasuo Mori¹

¹Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan

²Inserm U607, Laboratoire Canaux Calciques, Fonctions et Pathologies, 17 Rue des Martyrs, Bâtiment C3, 38054 Grenoble Cedex 09, France

³Department of Oral Biology, Graduate School of Dentistry, Tohoku University, Sendai 980-8575, Japan

⁴Howerd Hughes Medical Institute and Department of Molecular Physiology and Biophysics, Internal Medicine, and Neurology, University of Iowa Roy J. and Lucille A. Carver College of Medicine, Iowa City, Iowa 52242, USA

Correspondence and requests for materials should be addressed to Y.M.

(Tel: 81-75-383-2761, Fax: 81-75-383-2765, E-mail: mori@sbchem.kyoto-u.ac.jp)

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Abstract

Genetic analyses have revealed an association of the gene encoding the Rab3A-interacting molecule (RIM1) with an autosomal dominant cone-rod dystrophy CORD7. However, the pathogenesis of CORD7 has remained unclear. Recently, we have revealed that RIM1 exerts functional impacts on voltage-dependent Ca^{2+} channel (VDCC) currents and anchors neurotransmitter-containing vesicles to VDCCs, controlling neurotransmitter release. On the basis of this study, we demonstrate here that the mouse RIM1 arginine-to-histidine substitution (R655H), which corresponds to the human CORD7 mutation, modifies RIM1 function in regulating VDCC currents elicited by the P/Q-type $\text{Ca}_v2.1$ and L-type $\text{Ca}_v1.4$ channels. Thus, we can raise an interesting possibility that CORD7 phenotypes including retinal deficits and enhanced cognition are at least partly underlaid by altered regulation of presynaptic VDCC currents.

Originally identified as a putative effector for the synaptic vesicle protein Rab3, RIM1 is expressed in the brain and retinal photoreceptors where it is localized to the presynaptic ribbons in ribbon synapses.¹ RIM1 interacts with other presynaptic active zone protein components, including Munc13, ELKS (also known as CAST), RIM-BP and liprins, to form a protein scaffold in the presynaptic nerve terminal.²⁻⁶ Studies of mouse knockouts revealed that, in different types of synapses, RIM1 is essential for Ca²⁺-triggered neurotransmitter release as well as different forms of synaptic plasticity.^{5,7,8}

A mutation has been identified for an autosomal dominant cone-rod dystrophy CORD7 in the *RIM1* gene that is localized to chromosome 6q14.⁹ A four-generation British family with CORD7 first became aware of reduced color vision and visual acuity between the ages of 20 and 40 years. As the disorder progressed, they showed difficulty seeing in bright light, and one individual reported visual problems in dim light. At the onset of symptoms, retinal pigmentary changes were already present around the fovea, simulating a bull's eye dystrophy, which developed to macular atrophy.¹⁰ Interestingly, the affected individuals also showed significantly enhanced cognitive abilities across a range of domains.¹¹ Thus, the family with the CORD7 RIM1 mutation is characterized by retinal dystrophy as well as by enhanced brain function. However, mechanisms underlying these two phenotypes are yet to be elucidated.

Recently, we have revealed a previously unknown interaction between two components of the presynaptic active zone, RIM1 and VDCCs¹², that are essential for Ca²⁺-triggering of neurotransmitter release.¹³ RIM1 associates with VDCC β -subunits via its C terminus to markedly suppress voltage-dependent inactivation among different

neuronal VDCCs. In addition, membrane docking of vesicles is enhanced also by RIM1. Consistently, in pheochromocytoma neuroendocrine PC12 cells and in cultured cerebellar neurons, neurotransmitter release is significantly potentiated by RIM1. Thus, RIM1 association with VDCC β in the presynaptic active zone supports release via two mechanisms: sustaining Ca^{2+} influx through inhibition of channel inactivation, and anchoring neurotransmitter-containing vesicles in the vicinity of VDCCs. On the basis of this study, we specifically test here whether the RIM1 mutation associated with CORD7 affects regulatory effects of RIM1 on inactivation properties of VDCC currents, to gain insights in molecular mechanisms that underly the two phenotypes in CORD7 patients.

The mouse RIM1 mutation which corresponds to human CORD7 mutation R844H⁹, is a nucleotide (G to A) substitution that replaces Arg-655 with His in the middle C₂A domain (Fig. 1A), reported previously for its ability to bind to VDCC α_1 -subunit.⁶ The mouse clone differs from the human clone in having a deletion in the region between the Zn^{2+} -finger-like and PDZ domains. In this region, any specific functional domains have not been identified so far. Coimmunoprecipitation experiments suggest an intact interaction between RIM1 mutant R655H and the VDCC β_{4b} -subunit (Fig. 1B). To elucidate the functional effect of the RIM1 mutant, we characterized whole-cell Ba^{2+} currents through recombinant VDCCs expressed as $\alpha_1\alpha_2/\delta\beta_{4b}$ complexes containing neuronal α_1 -subunits $\text{Ca}_v2.1$ of P/Q-type VDCCs or $\text{Ca}_v1.4$ of L-type VDCCs. These VDCCs were selected, because P/Q-type $\text{Ca}_v2.1$ plays an important role in neurotransmitter release from central neuron¹³, while L-type $\text{Ca}_v1.4$ is found at high densities in photoreceptor terminals^{14,15} and known for its association with X-linked congenital stationary night blindness.¹⁶ Compared to wild-type RIM1 (WT), R655H

elicited significantly increased non-inactivating component of P/Q-type $\text{Ca}_v2.1$ currents (from 0.30 ± 0.04 ($n = 9$) to 0.43 ± 0.03 ($n = 6$); $P < 0.01$) (Fig. 1C). Inactivation parameters of L-type $\text{Ca}_v1.4$ current were unaffected by WT or by R655H (Fig. 1D) (see Table 1 for the estimated half-inactivation potential and the slope factor). In terms of activation properties, activation curve was shifted toward negative potentials (see Table 1 for the voltages for half-maximal activation and slope factors) and activation speed was increased by R655H in P/Q-type ($\text{Ca}_v2.1$) currents (Fig. 2A). Furthermore, RIM1-mediated augmentation of the current density in $\text{Ca}_v2.1$ currents was significantly enhanced by R655H (Fig. 2B) (see Table 1 the average of current densities). In contrast to $\text{Ca}_v2.1$ currents, $\text{Ca}_v1.4$ currents showed RIM1-mediated hyperpolarizing shift of activation curve abolished by the mutation (Fig. 2C). Effects of R655H on activation speed (Fig. 2C) and current densities were indistinguishable from those of WT in $\text{Ca}_v1.4$ channels (Fig. 2D).

Here, we demonstrate that the mouse RIM1 mutant R655H, which carries the counterpart mutation for human CORD7, alters RIM1 function in regulating VDCC currents. In the P/Q-type $\text{Ca}_v2.1$ VDCC known for its important role in neurotransmitter release in central synapses, CORD7 mutation elicited acceleration of activation, and enhanced RIM1-mediated suppression of inactivation and augmentation of current density, likely leading to an enhancement of neurotransmitter release and synaptic transmission. By contrast, in the L-type $\text{Ca}_v1.4$ VDCC known for its predominant role in glutamate release from photoreceptor terminals, the CORD7 mutation abolished the RIM1-mediated hyperpolarizing shift of activation curve, likely resulting in impaired synaptic transmission in ribbon synapses of the visual system. These different effects of CORD7 mutation on different presynaptic VDCCs may

eventually lead to the two reported phenotypes, retinal dystrophy and enhanced cognitive abilities, of distinct nervous tissues.

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Figure legend

Figure 1 CORD7 mutation R655H affects regulation of Ca_v2.1 channel inactivation by RIM1 via β association.

(A) Amino acid sequence alignment of C₂A domains of human, mouse and rat RIM1. The position of the CORD7 substitution (H) is indicated. (B) Physical association of recombinant β_{4b} and RIM1 mutant in HEK293 cells. The interaction is evaluated by immunoprecipitation (IP) with anti-FLAG antibody, followed by western blotting (WB) with anti-RIM antibody. Left panel: co-immunoprecipitation of wild-type RIM1 (WT) with FLAG- β_{4b} . Right panel: co-immunoprecipitation of R655H with FLAG- β_{4b} . (C) Effects of WT and R655H on the inactivation properties of P/Q-type Ca_v2.1 currents in BHK cells co-expressing α_2/δ and β_{4b} . Left panel: inactivation of P/Q-type Ca_v2.1 currents. The peak amplitudes are normalized for Ba²⁺ currents elicited by 2-s pulses to 0 mV from a holding potential (V_h) of -100 mV before and after expression of WT or R655H. Right panel: inactivation curves of P/Q-type Ca_v2.1 currents in BHK cells co-expressing α_2/δ and β_{4b} . The voltage dependence of inactivation, determined by measuring the amplitude of the peak currents evoked by 20-ms test pulses to 0 mV following 2-s prepulses to potentials from -100 to 20 with increments of 10 mV from a V_h of -100 mV, was fitted with the Boltzmann's equation. (D) Effects of WT and R655H on the inactivation properties of L-type Ca_v1.4 currents in BHK cells co-expressing α_2/δ and β_{4b} . Left panel: inactivation of L-type Cav1.4 currents. Right panel: inactivation curves of L-type Ca_v1.4 currents in BHK cells co-expressing α_2/δ and β_{4b} . The voltage dependence of inactivation, determined as in (C) above.

Figure 2 CORD7 mutation R655H affects regulation of Ca_v1.4 channel activation

by RIM1 via β association.

(A) Effects of WT and R655H on activation properties of $\text{Ca}_v2.1$ currents in BHK cells co-expressing α_2/δ and β_{4b} . Left panel: effects of WT and R655H on activation curves of $\text{Ca}_v2.1$ currents. Tail currents elicited by repolarization to -60 mV after 5-ms test pulse from -50 to 50 mV are used to determine activation curves. Right panel: effects on activation speed of $\text{Ca}_v2.1$ channels. Time constants are obtained by fitting the activation phase of currents elicited by 5-ms test pulse from -30 mV to 45 mV with a single exponential function. **(B)** Effects of WT and R655H on P/Q-type $\text{Ca}_v2.1$ current amplitude. Left panel: representative traces for Ba^{2+} currents evoked by test pluses from -40 mV to 50 mV with 10-mV increments in BHK cells co-expressing α_2/δ and β_{4b} . Right panel: current density-voltage (I - V) relationships of $\text{Ca}_v2.1$. The V_h is -100 mV. **(C)** Effects of WT and R655H on activation properties of $\text{Ca}_v1.4$ currents in BHK cells co-expressing α_2/δ and β_{4b} . Left panel: effects of WT and R655H on activation curves of $\text{Ca}_v1.4$ currents. Right panel: effects on activation speed of $\text{Ca}_v1.4$ channels. **(D)** Effects of WT and R655H on L-type $\text{Ca}_v1.4$ current amplitude. Left panel: representative traces for Ba^{2+} currents on application of test pluses from -40 mV to 50 mV with 10-mV increments in BHK cells co-expressing α_2/δ and β_{4b} . Right panel: I - V relationships of $\text{Ca}_v1.4$. The V_h is -100 mV.