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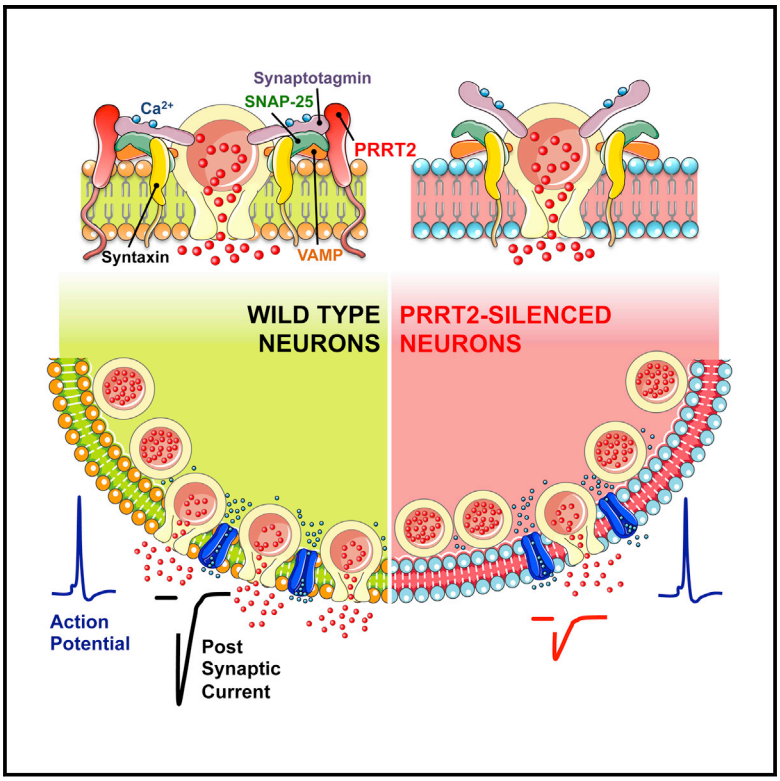
PRRT2 Is a Key Component of the Ca^{2+}-Dependent Neurotransmitter Release Machinery

Graphical Abstract



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In Brief

Valente et al. show that PRRT2, a single causative gene for a group of paroxysmal neurological diseases, is a key component of regulated exocytosis. Silencing PRRT2 dramatically impairs neurotransmitter release by markedly reducing release probability. PRRT2 interacts with the fast Ca^{2+} sensors synaptotagmin 1/2 and endows the SNARE complex with Ca^{2+} sensitivity.

Highlights

- PRRT2 is a presynaptic protein
- PRRT2 is required for synchronous neurotransmitter release
- PRRT2 silencing decreases synaptic density and release probability
- PRRT2 interacts with the fast Ca^{2+} sensors synaptotagmin 1/2

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PRRT2 Is a Key Component of the Ca^{2+}-Dependent Neurotransmitter Release Machinery

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SUMMARY

Heterozygous mutations in proline-rich transmembrane protein 2 (PRRT2) underlie a group of paroxysmal disorders, including epilepsy, kinesigenic dyskinesia, and migraine. Most of the mutations lead to impaired PRRT2 expression, suggesting that loss of PRRT2 function may contribute to pathogenesis. We show that PRRT2 is enriched in presynaptic terminals and that its silencing decreases the number of synapses and increases the number of docked synaptic vesicles at rest. PRRT2-silenced neurons exhibit a severe impairment of synchronous release, attributable to a sharp decrease in release probability and Ca^{2+} sensitivity and associated with a marked increase of the asynchronous/synchronous release ratio. PRRT2 interacts with the synaptic proteins SNAP-25 and synaptotagmin 1/2. The results indicate that PRRT2 is intimately connected with the Ca^{2+}-sensing machinery and that it plays an important role in the final steps of neurotransmitter release.

INTRODUCTION

Over the last 4 years, several studies have identified an array of heterozygous nonsense, frameshift, and missense mutations in the gene encoding proline-rich transmembrane protein 2 (PRRT2) in a large number of cases affected by different paroxysmal disorders such as benign familial infantile seizures, infantile convulsion choreoathetosis, migraine, hemiplegic migraine, paroxysmal kinesigenic dyskinesia/choreoathetosis, benign familial infantile seizures/epilepsy, and episodic ataxia (Chen et al., 2011; Lee et al., 2012; for review, see Ebrahimi-Fakhari et al., 2015). The astonishing pleiotropy of the phenotypic expression of PRRT2 mutations points to an overlap in the pathogenic pathways and to a very important role of this gene in regulating synaptic transmission and network activity.

The PRRT2 gene is located in human chromosome 16 and consists of four exons encoding a poorly characterized protein of 340 amino acids. Most mutations identified in PRRT2 are phenotypically highly penetrant and cause truncation of the protein because of nonsense or frameshifts mutations that result in mRNA degradation by nonsense-mediated mRNA decay or degradation of the protein (Wu et al., 2014), suggesting a loss-of-function mechanism of action.

PRRT2 mRNA has been shown to be almost exclusively expressed in neurons in the cortex, hippocampus, basal ganglia, and cerebellum (Chen et al., 2011; Lee et al., 2012; Heron et al., 2012; Trabzuni et al., 2011), which are all regions putatively involved in the pathogenesis of the PRRT2-linked diseases. The functional role of this protein is totally unknown. A yeast two-hybrid screen highlighted a potential interaction of PRRT2 with synaptosomal-associated protein 25 kDa (SNAP-25), one of the presynaptic soluble N-ethylmaleimide sensitive factor (NSF) attachment protein receptor (SNARE) proteins triggering fusion of synaptic vesicles (SVs) (Stelzl et al., 2005; Lee et al., 2012). Recently, PRRT2 has also been found among proteins associated with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors by a high-resolution proteomics study (Schwenk et al., 2014), and its mutation was associated with increased levels of extracellular glutamate (Li et al., 2015).

To investigate the role of PRRT2 in presynaptic physiology, we silenced PRRT2 expression in primary neurons using RNA interference. PRRT2 knockdown caused a marked impairment in synaptic transmission because of a decrease in the density of synaptic connections and a sharp reduction in release probability. Both effects were fully reversible upon re-expression of shRNA-resistant PPRT2. In addition to SNAP-25, the search for PRRT2 partners identified the fast Ca^{2+} sensors synaptotagmins 1/2, responsible for triggering synchronous neurotransmitter release. The results indicate that PRRT2 is intimately connected with the Ca^{2+}-sensing machinery of neurotransmitter release.

RESULTS

PRRT2 Is a Presynaptic Protein Developmentally Expressed during Synaptogenesis

We evaluated the regional expression of PRRT2 protein in various areas of the adult mouse brain and the developmental