Familial hemiplegic migraine type-1 R192Q knock-in mice exhibit altered neuromuscular synaptic transmission

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Mutations of the CACNA1A gene, encoding the α1A (Cav2.1) pore-forming subunit of the P/Q-type calcium channel, cause a wide spectrum of diseases, including familial hemiplegic migraine type-1 (FHM1).

P/Q-type calcium channels are expressed pre-synaptically at both central synapses and the peripheral neuromuscular junction (NMJ), where they mediate neurotransmitter release. Thus, Cav2.1 mutations are likely to induce synaptic malfunction. This hypothesis is supported by previous studies from our laboratory revealing synaptic defects at NMJs of the spontaneous Cav2.1 mutants *tottering*, *Rolling Nagoya* and *leaner*.

We generated transgenic mice carrying the human FHM1 mutation R192Q. Heterozygous and homozygous mice are viable and do not show an overt clinical phenotype.

Ex vivo electrophysiological analysis of NMJs of R192Q mice showed 155% and 65% increase in spontaneous quantal acetylcholine (ACh) release in homozygous and heterozygous knock-in mice, respectively, compared with controls. Slight depolarization by 10 mM K+ increased spontaneous uniquantal release at wild-type NMJs approximately 8-fold. At R192Q NMJs, however, the increase was more pronounced, about 14-fold.

Low-rate (0.3 Hz) evoked ACh release was found unaltered at R192Q NMJs. At low (0.2 mM) extracellular calcium, however, evoked release was increased by ~240% and ~110% in homozygous and heterozygous mice, respectively, compared to wild-type. High-rate (40 Hz, 1 s) release showed slightly more pronounced rundown in the initial phase of the train at R192Q NMJs.

We investigated *in vivo* neuromuscular transmission by carrying out grip strength measurements and, in anaesthetized mice, electromyographical recordings. None of the *in vivo* parameters determined was affected in young or aged homozygous R192Q mice.

Detailed light- and electron-microscopical analyses did not reveal any morphological abnormalities underlying the observed *ex vivo* electrophysiological changes.

Our results strengthen the hypothesis that synaptic dysfunction contributes to FHM1 disease symptoms, and show that R192Q mutant mice are valuable for FHM1-related pathophysiological studies.

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