Motor nerve terminals with fast downregulation of Syt1 are more vulnerable in Spinal Muscular Atrophy

Spinal Muscular Atrophy (SMA) is the most frequent genetic cause of infant mortality. It is caused by mutations or loss of the *SMN1* gene, which codifies for the Survival of Motor Neuron (SMN) protein. SMN has an important role in pre-mRNA maturation. The disease progression follows a pattern of neuromuscular impairment in which some groups of muscles are more affected than others, and a few are not affected at all. The basis for this selective vulnerability is, however, unknown.

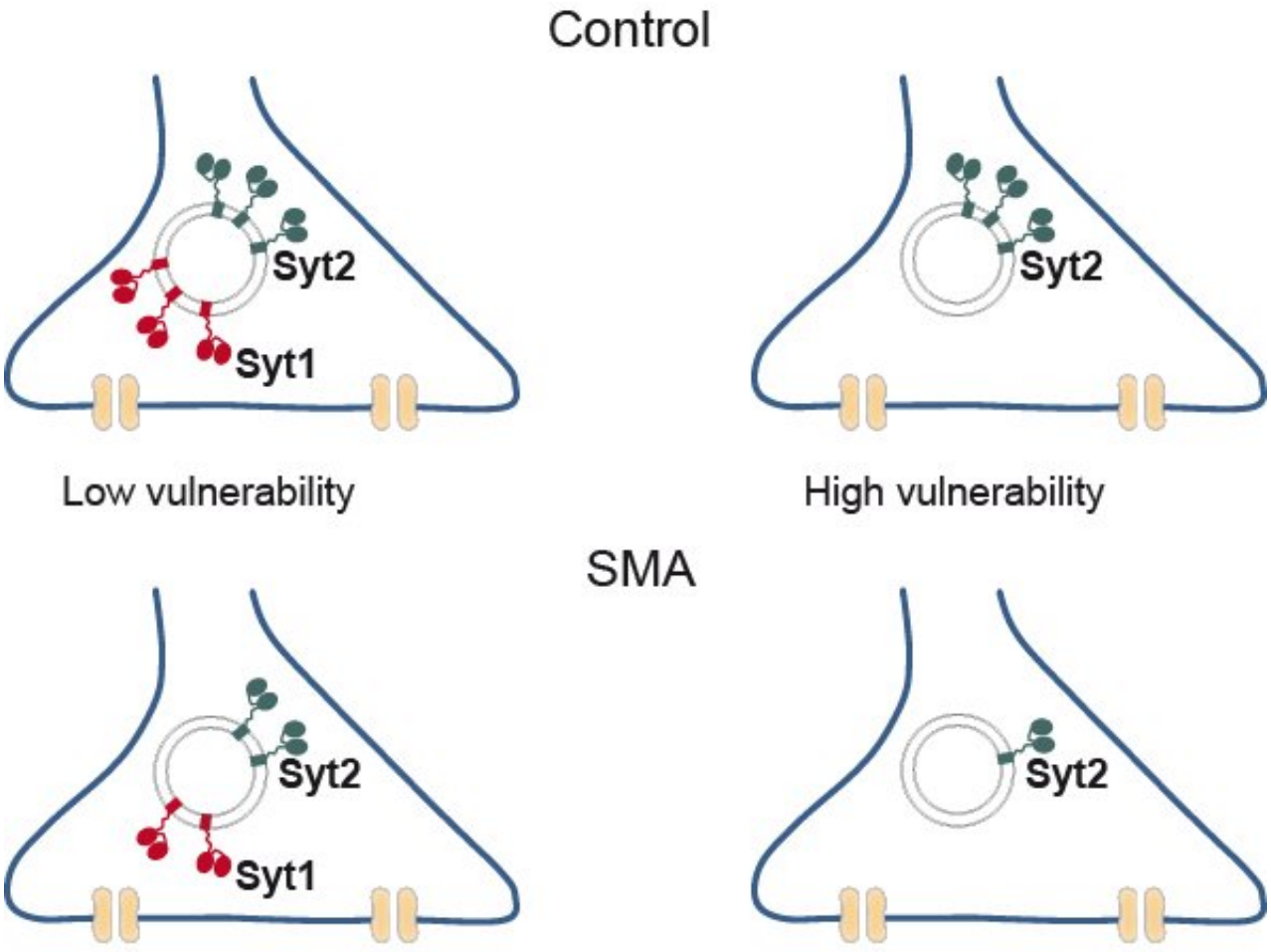

Fig. 1. Synaptotagmin contents in motor nerve terminals of low and high vulnerable muscles in control and SMA mice. In control, at P9, both isoforms are co-expressed in low vulnerable muscles, but only Syt2 is detected in high vulnerable muscles. In SMA mice, Syt2 is decreased in all muscles, but more in terminals of vulnerable muscles. Syt1 and Syt2 levels are represented by arbitrary copy numbers in relative proportion to their expression.

In a mouse model of SMA, evoked neurotransmitter release is decreased in the motor nerve terminals of certain muscles, but normal in others. We investigated with electrophysiological and quantitative immunofluorescence methods the possible molecular determinant of the impairment by comparing the postsynaptic responses and the level of presynaptic proteins in control and SMA high vulnerable (*Transversus abdominis*, *Oblique internus abdominis*) and low vulnerable (*Levator auris longus*, diaphragm) muscles.

We found that, at postnatal day nine, synaptotagmin 2 (Syt2), the calcium sensor for neurotransmitter release in mature motor neurons, inhibitory interneurons, and calyx of Held, is highly decreased in SMA motor nerve terminals in comparison with controls, especially in more vulnerable muscles (Fig. 1). In addition, the expression level of Syt1, the predominant fast calcium sensor for exocytosis in forebrain neurons, is inversely correlated with muscle vulnerability (Fig. 1). Together, these results evidence that both isoforms are particularly low in SMA vulnerable muscles (Fig. 1). Furthermore, a synaptic protein that interacts with Syt1 and Syt2, the synaptic vesicle protein 2 (SV2) B, is also reduced in direct proportion with muscle vulnerability in SMA nerve terminals, but not syntaxin 1B and synaptotagmin 7.

By investigating the expression of Syt2 and Syt1 at different ages, we found that they are developmentally regulated. Syt1 is present at the embryonic stage, Syt1 and Syt2 coexist during the postnatal maturation period, and Syt2 is expressed at the mature stage. Remarkable, the physiological downregulation of Syt1 is faster in the nerve terminals of more vulnerable muscles (Fig. 2). These findings suggest that the decrease in Syt2 in SMA terminals is not compensated by Syt1 in synapses that *mature* sooner.

What are the functional consequences of the synaptotagmins deficit? Syt1 and Syt2 participate in vesicle docking, positional priming, and endocytosis, and synchronous synaptic transmission is severely impaired in Syt1 and Syt2 knockouts. In fact, the synaptic alterations in SMA neuromuscular synapses in the electrophysiological recordings are comparable to the functional phenotypes of Syt2-deficient mice.

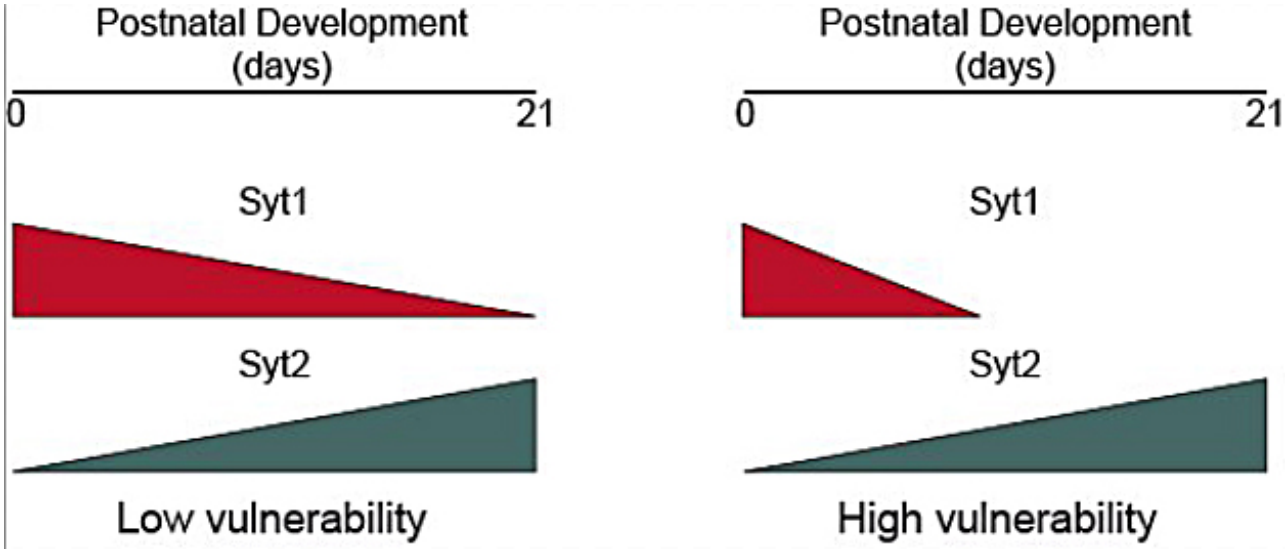

Fig. 2. Physiological postnatal regulation of calcium sensors in motor nerve terminals. During postnatal maturation, Syt1 and Syt2 expression are inversely regulated. In less affected SMA muscles, Syt1 downregulation is slower than in high vulnerable muscles.

Thus, our study indicates that the fast physiological decrease of Syt1 in some muscles, together with the pathological reduction of Syt2, are important molecular determinants of muscular selective vulnerability, and greatly explain the functional deficit at the neuromuscular junction in the SMA disease mouse model.

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