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General Article

GENERAL ARTICLE

Fragile X premutation rCGG repeats impair synaptic growth and synaptic transmission at *Drosophila* larval neuromuscular junction

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Abstract

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disease that develops in some premutation (PM) carriers of the *FMR1* gene with alleles bearing 55–200 CGG repeats. The discovery of a broad spectrum of clinical and cell-developmental abnormalities among PM carriers with or without FXTAS and in model systems suggests that neurodegeneration seen in FXTAS could be the inevitable end-result of pathophysiological processes set during early development. Hence, it is imperative to trace early PM-induced pathological abnormalities. Previous studies have shown that transgenic *Drosophila* carrying PM-length CGG repeats are sufficient to cause neurodegeneration. Here, we used the same transgenic model to understand the effect of CGG repeats on the structure and function of the developing nervous system. We show that presynaptic expression of CGG repeats restricts synaptic growth, reduces the number of synaptic boutons, leads to aberrant presynaptic varicosities, and impairs synaptic transmission at the larval neuromuscular junctions. The postsynaptic analysis shows that both glutamate receptors and subsynaptic reticulum proteins were normal. However, a high percentage of boutons show a reduced density of Bruchpilot protein, a key component of presynaptic active zones required for vesicle release. The electrophysiological analysis shows a significant reduction in quantal content, a measure of total synaptic vesicles released per excitation potential. Together, these findings suggest that synapse perturbation caused by riboCGG (rCGG) repeats mediates presynaptically during larval neuromuscular junction development. We also suggest that the stress-activated c-Jun N-terminal kinase protein Basket and CIDE-N protein Drep-2 positively mediate Bruchpilot active zone defects caused by rCGG repeats.

Introduction

The fragile X mental retardation 1 (*FMR1*) gene normally harbors a highly polymorphic trinucleotide repeat sequence (CGG) within its 5′ untranslated region (5′ UTR). The normal allele of the *FMR1* gene typically has 5–40 CGG repeats. Abnormal alleles include the full mutation (>200 CGG repeats), premutation (PM) (55–200 CGG repeats) and gray zone mutation (45–54 CGG repeats). Carriers of full mutation develop fragile X syndrome (FXS; OMIM: 300624), the most common inherited form of neurodevelopment and intellectual disability (ID) disorder, occurring in 1 in 4000 to 1 in 7000 people (1–4). On the other hand, PM carriers account for a variety of phenotypes that are found frequently in the population, with an estimated prevalence of 1:259 in females and 1:813 in males (5,6). A proportion of these PM carriers, about 40% of males and 16% of females develop a

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