Abstract

Pathogenic mutations in the NALCN channel complex cause the severely debilitating diseases CLIFAHDD and IHPRF1/2, which are ultra-rare by affecting only around 100 patients worldwide. NALCN is an ion channel that is widely expressed in the brain, and carries a basal cationic current across neuronal membranes. This constitutively active current affects the voltage across the membrane, determining cell excitability and regulating electric signalling. Correct signal propagation between the central and peripheral nervous systems is of high physiological importance for basic bodily functions, such as breathing and motor control. Disease-causing mutations are disruptive to these processes and result in respiratory problems, lack of muscle control, and severe global developmental and intellectual delay. Recent studies have provided new insight into NALCN structure and function, showing the assembly of NALCN, UNC80, UNC79 and FAM155A to form a large channel complex. These advancements allow the systematic study of pathogenic mutations in NALCN and UNC80 that cause CLIFAHDD/IHPRF1 and IHPRF2, respectively. Here we show that CLIFAHDD mutations exhibit gain-of-function phenotypes when expressed in *Xenopus* oocytes and HEK293T cells. The CLIFAHDD mutations cluster around the pore-forming segments of the NALCN channel, likely increasing open probability by destabilizing the pore. In contrast, protein-truncating IHPRF1/2 nonsense mutations in NALCN and UNC80 cause a complete loss of function. Although most tested mutations show distinctive gain- or loss-of-function phenotypes, a few notable exceptions prove the limitations of validating functional effects through heterologous expression. Using these methods, we were unable to assess putative trafficking defects, changes in post-translational modifications and protein-protein interactions, and these parameters will require further studies, e.g. in induced human pluripotent stem cells or animal models. In summary, this work provides an overview of the functional effects of CLIFAHDD/IHPRF mutations on the NALCN complex and the results may ease future therapeutic approaches.