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Biophysical characterization of a novel gene mutation associated with the X-linked Charcot-Marie-Tooth disease using light-based techniques

Connexin-32 (Cx32), encoded by the *GJB1* gene, play a crucial role in communication between living cells. Mutations in this gene lead to the X-linked form of Charcot-Marie-Tooth (CMT1X) disease, a progressive neuropathy that damages peripheral nerves. We identified a novel gene mutation (H73L) located at the border between the Cx32 protein's extracellular loop and the transmembrane domain. This site is hypothesized to be critical for the assembly and docking of connexin hemichannels, as well as for their voltage and calcium-dependent gating. Functional experiments were performed at the populational level by a luciferase/luciferin assay based on luminescence, revealing a decrease in both calcium-dependent opening and closure and a leakier hemichannel in cells with the H73L mutation. These results were confirmed by fluorescent dye uptake, and IP3 flash photolysis at the single-cell level using an ATP biosensor. Molecular dynamics simulations of the mutant hemichannel supported our experimental findings, revealing that the H73L mutation weakens electrostatic interactions in a critical area for calcium-dependent gating, accompanied by a global widening of the pore, potentially leading to increased channel permeability. Overall, these findings expand our understanding of *GJB1* gene mutations associated with CMT1X and suggest that dysfunction of Cx32 hemichannels may contribute to the disease.

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