Determining a link between XLID and missense mutations in Chloride Intracellular Ion Channel Protein 2 (CLIC2): a biophysical approach

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The Chloride Intracellular Channel (CLIC) protein family belongs to a growing class of proteins called metamorphic proteins. They have the unique dynamic ability to reversibly auto-insert into membranes to form active ion channels. A recent large-scale next generation resequencing of the X chromosome revealed several missense mutations (nsSNPs) in the CLIC2 gene of healthy individuals (S109C, P160A, D161H & D161Y). This indicates that the CLIC2 protein can be functional notwithstanding these mutations. Furthermore, a H101Q mutation was identified in a male with an X-linked intellectual disability (XLID). Recently, an *in silico* modelling study anticipated that the structural stability, inherent flexibility and membrane-binding characteristics of CLIC2 are affected by these mutations. It was proposed that the H101Q disease linked mutation, located in a key hinge region of CLIC2, made the CLIC2 protein more stable in solution, which inhibited its plasticity required for membrane insertion. To verify the modelled findings, *in vitro* experiments are being conducted on mutants H101Q, S109C, P160A & D161H to determine the thermal stability and membrane-binding properties of the CLIC2 protein. The missense mutations have been introduced into the cloned CLIC2 proteins. Circular dichroism and differential scanning fluorimetry were then conducted on the expressed and purified proteins in order to help analyze their structural and thermal stability. The results gain importance in light of *in silico* modeling studies which suggest that the H101Q mutation modifies the interaction of CLIC2 with the membrane.