# Functional Studies of a Mutation in the Nicotinic Acetylcholine Receptor That Leads to a Congenital Myasthenic Syndrome


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## PURPOSE:

The long-term goal of this project is to understand how a mutation, which leads to a Congenital Myasthenic Syndrome, influences the activity of the nicotinic acetylcholine receptor. A prokaryotic homologue, ELIC, is used to study the effects of this lipid-facing mutation in the M4 transmembrane α-helix on channel gating. Specifically, I used electrophysiological methods to characterize the activity of the L308C ELIC mutant.

## INTRODUCTION:

The nicotinic acetylcholine receptor (nAChR) is a neurotransmitter-gated ion channel central to signal transmission at the neuromuscular junction (Fig. 1).

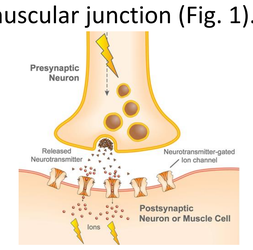


**Figure 1. The synapse at the neuromuscular junction.**

A αC418W mutation in the nAChR leads to a Congenital Myasthenic Syndrome (CMS) (Fig. 2).

**Figure 2. An individual suffering from a CMS.** Many symptoms are associated with muscle weakness especially in the extremities and eyes.

The αC418 is a lipid-facing residue located on the M4 transmembrane α-helix (Fig. 3). I hypothesized that the αC418W mutation promotes binding of M4 to the adjacent M1 and M3 α-helices, which in turn promotes the coupling of protein binding and gating. The goal here is to test this hypothesis using the prokaryotic homolog, ELIC, for which a crystal structure is known.

Here, I characterize a Leu to Cys mutation at position 308 in ELIC – i.e. the position that corresponds to position 418 in the nAChR. Consistent with my hypothesis, I show that the chemistry at this site influences channel gating. Future experiments will characterize the L308W mutation. The long term goal is to place the L308C and L308W mutations on a background where M4 binding to M1/M3 is already optimized. This will allow me to test my hypothesis that this disease causing mutation alters M4 binding with M1 and M3.

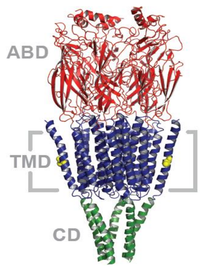


**Figure 3. Structure of the *Torpedo* nAChR.** Yellow spheres show the location of the αC418 residue. ABD: agonist binding domain, TMD: transmembrane domain, CD: cytoplasmic domain

## METHODOLOGY:

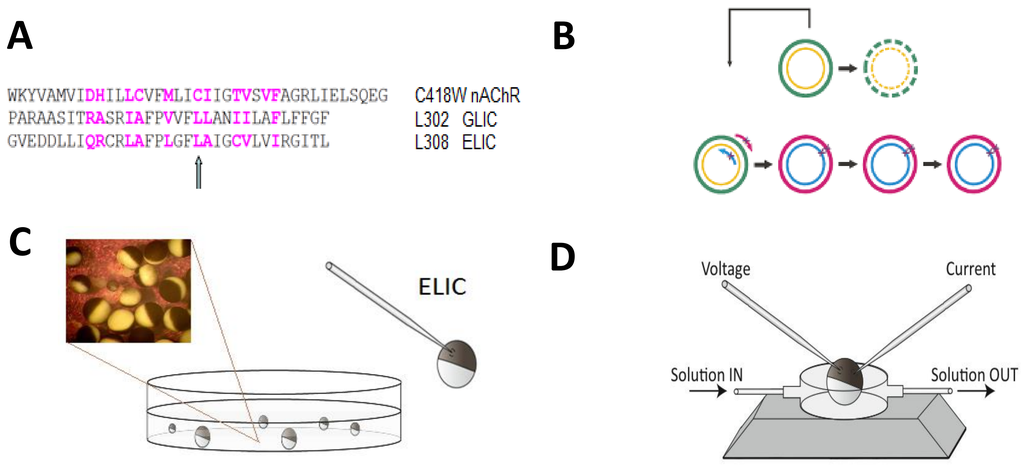


**Figure 4. Measurement of electrical activity of ELIC. a)** Sequence comparison between *Torpedo* nAChR, GLIC and ELIC. **b)** PCR mutagenesis to incorporate L308C mutation in ELIC. **c)** 0.1ng of ELIC RNA is injected in Stage V-VI *Xenopus laevis* frog oocytes and incubated for 24 hours at 16°C . The oocytes are kept in a 1X ND96+ buffer with Kanamycin for optimal oocyte health. **d)** Two-electrode Voltage Clamp apparatus (TEVC). ELIC is then exposed to varying concentrations of the agonist, cysteamine, in 150mM NaCl, 10mM HEPES, 0.5mM BaCl, 100mM DTT.

## RESULTS:

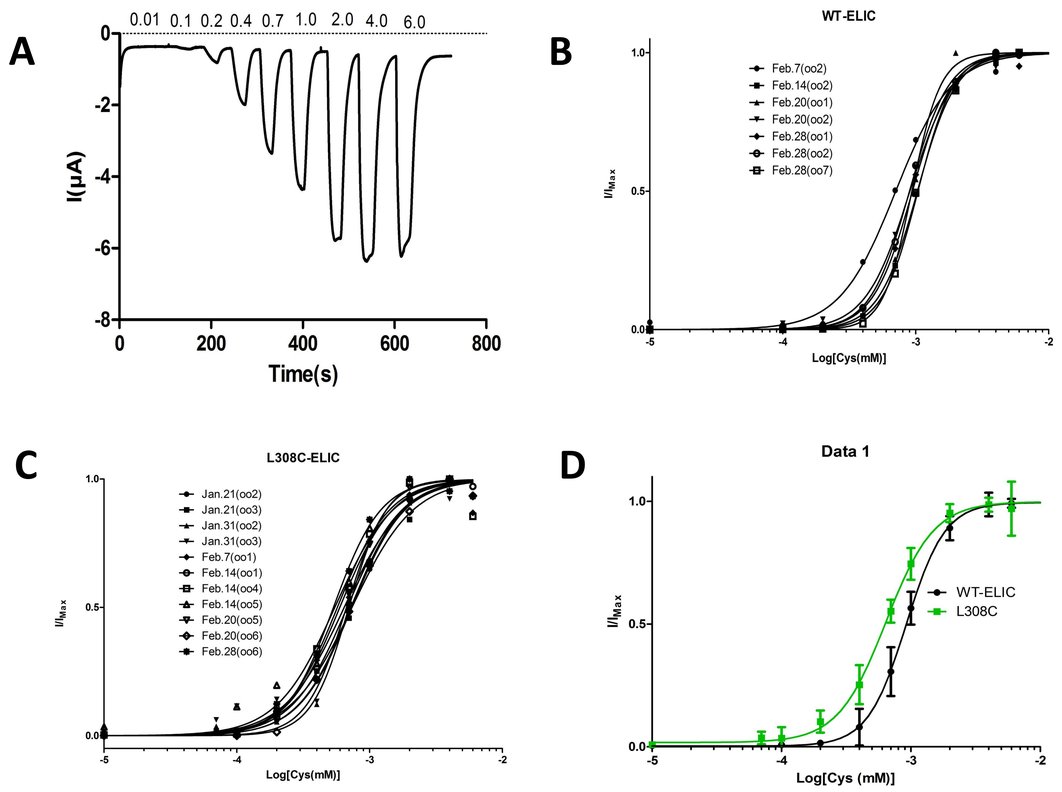


**Figure 5. Characterization of the L308C mutation. a)** Whole cell TEVC recordings in response to the noted concentrations of cysteamine. **b)** Cysteamine dose response curves for wild type-ELIC. **c)** Cysteamine dose response curves for the L308C mutation. **d)** Comparison of the average dose response curves of wild type-ELIC and L308C. The calculated $EC_{50}$ for WT and L308C are 0.9228mM and 0.6411mM respectively. **Because the mutation is distant from the agonist site (Fig. 3), it can be concluded that the L308C mutant shows increased coupling of binding and gating.**

## DISCUSSION:

This gain of function as a result of the L308C mutation suggests increased efficiency in the coupling of agonist binding to channel gating, showing that this lipid-exposed site on M4 is a determinant of channel gating.

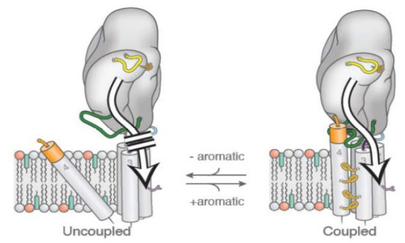


**Figure 6. Proposed mechanism showing how M4 structure may influence channel gating.**

## FUTURE IMPLICATIONS:

Results obtained with the L308C mutant act as a control to allow for the comparison with the L308W mutation, the substitution from leucine to tryptophan in ELIC. It is hypothesized that the tryptophan residue will not interact favorably with lipids, thus promoting interactions between M4 and M1/M3 in a manner that promotes interactions between the agonist binding and channel gating domains.

To further test this allosteric coupling mechanism in ELIC, both mutations will then be placed on a background where the M4 and M1/M3 α-helices are already tightly bound to each other. To do this, aromatic residues will be added to the M1/M3/M4 interface (Carswell et al. 2014). If the hypothesis is correct, then the L308W mutation on this maximally coupled background should not have any further effects on channel gating.

## ACKNOWLEDGEMENTS:


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## REFERENCES:


Carswell CL, Therien JPD, Juranka PF, Surujballi JA, Baenziger JE. Intramembrane aromatics facilitate gating and govern the susceptibility of pentameric ligand-gated ion channels to potentiation. Under revision.

daCosta CJB, Baenziger JE (2009) A lipid-dependent uncoupled conformation of the acetylcholine receptor. J Biol Chem 284: 17819-17825

Shen XM, Deymeer F, Sine SM, Engel AG (2006) Slow-channel mutation is acetylcholine receptor αM4 domain and its efficient knockdown. Ann Neurol 60:128-136