### Abstract

- **Rabies virus is known to bind to nicotinic acetylcholine receptors (nAChR)**
- **The virus binding region has been characterized in alpha 1 subunits of these receptors**
- Little research has been done on the other alpha subunits
- Understanding binding region sequences can help understand virus adaptation to host species
- Using primers based on dog sequences, the proposed binding region of alpha subunits were amplified and sequenced
- **Current Results:**
  - **Red fox and dog have nearly identical alpha 1, 2, 3, 5, and 6 sequences for the proposed binding region**
  - **Arctic fox has nearly identical sequences to dog and red fox in alpha 1, 2, and 6**
  - **Raccoon’s alpha 1, 2, 3, 5, and 6 subunits are similar enough to allow for annealing of the dog-based primers in PCR**
  - No clear PCR products were found for skunk DNA
- **Future goals:**
  - Sequencing five individuals per species
  - Adding mongoose, bat, and opossum

### Introduction

- **Rabies is known to infect all mammals and is adapted to different host species**
- Has a 100% fatality rate in infected individuals
- 50,000 humans are killed worldwide, mostly in Asia and Africa
- Rabies virus surface protein shows similarity to snake toxins which are known antagonists of nAChR (Figure A, C, and D)
- The virus binding region has been characterized in alpha 1 subunits in previous research
- Little research has been done on the binding of rabies to other nAChR subunits, especially those present in the central nervous system
- Understanding how rabies interacts with these nervous system associated nAChR is of great interest for this disease infects the central nervous system

### Sequencing of rabies binding region on nicotinic acetylcholine receptor alpha subunits in four host species

**Methods**

- Primes were designed to amplify the predicted rabies virus binding region in nAChR alpha subunits 1 to 7 using known sequences from dogs (Canis familiaris)
- PCR was performed individually on each subunit using DNA from 4 host species: red fox (Vulpes vulpes), arctic fox (Vulpes lagopus), skunk (Mephitis mephitis), and raccoon (Procyon lotor)
- Amplification of the target region was confirmed using gel electrophoresis
- PCR product was then processed and the DNA sequenced
- Sequenced DNA was then analyzed using 4peaks and ClustalW2

**Results**

- **Tyr185 and Tyr192, important to snake neurotoxin binding to Lymnaea stagnalis acetylcholine receptor binding protein (AChBP), was conserved in all alpha subunits in red fox, arctic fox, dog, calf, and human except alpha 5, where Tyr184 was switched to Asp in all species (Figure B)**
- Several other residues, such as Asp194, Cys187, Cys188, and Ile174, are also highly conserved in most target regions in the species compared
- It is not known if these residues interact with the neurotoxin or rabies, but they may play a role since they are highly conserved
- **Red fox results**
  - Complete conservation compared to arctic fox and dog for alpha 1,2,3, and 5
  - Nearly identical compared to arctic Fox and dog for alpha 6, differing by 1 amino acid
  - Nearly identical sequences to human for alpha 1, 5, and 6; complete conservation in alpha 3
- Arctic fox results
  - Complete conservation between arctic fox and dog for alpha 1, 2, 3, and 5
  - Nearly identical to human for alpha 1 and 2, with 4 and 2 differences in amino acids respectively
  - Alpha 6 showed complete conservation between arctic fox and human
- **Raccoon results**
  - Target region showed enough similarity to dog sequence to allow primers to anneal; however, no clear sequences have been obtained
- **Skunk results**
  - Target region has not shown enough similarity to allow dog primer anneal; no usable PCR product has been obtained

**Discussion**

- Comparing the sequences of several species, the more closely related species, such as dog, arctic fox, and red fox, show a higher degree of conservation
- Species more distantly related, namely fox species and humans, show a greater degree of difference in amino acid sequence in the target region
- This difference in sequence between distantly related species could explain why species-crossover of rabies strains have difficulty maintaining endemic infections
- However the similarity in sequence between red fox and arctic fox does not explain the differences seen in their species-specific rabies strains
- More research will need to be conducted to elucidate differences between species

**Future Goals**

- Sequence 5 individuals of each species to compare sequences within species
- Add bat, mongoose, and opossum to study
- Of great interest is the opossum sequence, considering several studies have shown that opossums have a resistance to rabies infections not seen in other mammals

### References and Acknowledgements

- Lentz, TL. 1990. “Rabies virus binding to an acetylcholine receptor alpha-subunit peptide”. Journal of Molecular Recognition, 3(2): 82-88
- Special thanks to Dr. Christopher Kyle, Trent University, for the raccoon and skunk DNA

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**Figure A**: snake neurotoxin (gok) binding to the acetylcholine binding protein in Lymnaea stagnalis (yellow and blue), a structural homology to mammalian acetylcholine receptors found in mammals

**Figure B**: completed sequences of nAChR alpha subunits for several species. The L. stagnalis sequence shows the region known to bind to neurotoxin loop 2 in snake venom. The blue bar denotes the rabies binding region characterized in calf nAChR alpha 1 (sequence shown for alpha 1, blue dot). Outlined in black are residues found to be important for neurotoxin loop 2 binding to nAChR. Residues marked with ( ) show strong similar chemical properties. Residues marked with ( ) show weakly similar chemical properties. Notice that Tyr184 and Tyr192 are conserved in all subunits except in alpha 5, where Tyr184 was replaced with Aspartic Acid (D).

**Figure C**: Close up look at the bound neurotoxin peptide. Neurotoxin residues (black labels) interact with the complementary residues on the AChBP (blue labels). Areas of interaction are highlighted in yellow and cyan.

**Figure D**: Comparison of neurotoxic loop 2 for snake toxin and rabies. Bold residues are highly conserved between toxins and rabies. Residues circled red are known to interact with the acetylcholine binding protein (AChBP) when snake toxin is bound. Residues circled blue have been shown to be important in rabies binding to receptors.