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EVOLUTIONARY ANALYSES OF MAMMALIAN DOUBLE STRANDED RNA BINDING PROTEINS

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Background: Mammalian hosts engage in evolutionary arms races with their viral pathogens, where both the host and the virus are continuously competing with one another. We can uncover these arms races by comparing phylogenetic gene sequences, to test for increased missense changes in homologous species (dN/dS>1). Seeing results of dN/dS>1 are signs of positive selection, also referred to as rapid evolution.

Host double-stranded RNA binding proteins (dsRBPs) can recognize viral genomes *(6,5)*, leading to the initiation of host-protective immune responses. Previous research identified specific human dsRBPs with important antiviral roles, such as OAS1 *(3,4)* and strategies used by viruses to evade recognition by these host factors. Understanding the roles and evolutionary histories of these dsRBPs teaches us about pathogen vulnerabilities and how dysregulation of these genes can cause disease. Our research applies an evolutionary approach to the study of dsRBPs, using computational methods to analyze primate dsRBP orthologs for positive selection and reveal new evolutionary arms race based at the dsRBP-virus interface. We are focused on the TAR RNA binding protein (TRBP) and the melanoma differentiation associated gene 5 (MDA5), both which have shown antiviral roles.

Purpose: We hypothesize that if TRBP or MDA5 are part of an evolutionary arms race between primate hosts and their viral pathogens, then we will see evidence of positive selection in the TRBP and MDA5 genes of primates.

Methods: We aligned ≈20 simian sequences from GenBank, in Geneious, for TRBP and MDA5. Alignments were assessed for positive selection using PAML (Phylogenetic Analysis by Maximum Likelihood), to determine how well two scenarios fit the alignments: one scenario where there is no positive selection, and one where there is. We then used the Likelihood Ratio Test to find which scenario fit best, leading us to determine if our gene of interest is under positive selection. We then added prosimians to the primate tree, which increased tree length, and repeated this analysis.

To understand the contributions of particular species, we used PAML to run a branch-site test on MDA5 to determine the specific dN/dS values of each branch in our tree, which included the prosimians.

Results: TRBP showed no evidence of positive selection.

For MDA5, the simian analysis showed dN/dS = 0.26009 indicating positive selection, but the p-value, 0.2421913894 > 0.05, was not significant.

When we added prosimians, dN/dS = 2.15279, and p = 0.0002799935474 < 0.05. These calculations tell us that MDA5 shows evidence for positive selection occurring, and PAML identified 7 specific amino acid positions that are likely to be under positive selection.

Our branch-site analysis identified that this signal of rapid evolution came from the branch leading to the prosimian species, being the only branch with dN/dS > 1.

Conclusions: We observed positive selection for MDA5 in prosimians, but not in simians alone. This result supports our hypothesis that an arms race exists between prosimian hosts and RNA viruses, but further experiments are required to verify our hypothesis…

In the future, we will map the positively selected sites predicted by PAML onto MDA5's crystal structure *(1,7)* in order to generate hypotheses about how this positive selection might affect MDA5's ability to recognize and engage with dsRNA or other proteins. We will also analyze MDA5 in additional mammalian species, including rodents and bats *(2)*.

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