Epigenetic Analysis of the SLC6A4 Serotonin Transporter in Bottlenose Dolphins (*Tursiops truncatus*)

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Background

- The bottlenose dolphin (*Tursiops truncatus*), along with several other marine mammals, exhibit the atypical behavior of stranding, or better known as beaching.
- Studies have shown that early life stress causes epigenetic modifications to stress-related genes resulting in abnormal behavior in rodents and humans.
- Epigenetic modifications can be analyzed by looking at methylation of gene promoters.
- We hypothesize that stranding could be a stress-related response and methylation patterns will differ between stranded and non-stranded dolphins.
- In this project, we will be looking at methylation patterns in the promoter region of the serotonin transporter SLC6A4, a stress gene.
- The SLC6A4 gene encodes an integral membrane protein that transports serotonin from synaptic spaces into presynaptic neurons. The encoded protein terminates the action of serotonin and recycles it. This protein is a target of many antidepressant medications.

Objectives

- To better understand marine mammal stranding behavior by looking at epigenetic modifications (methylation) of stress genes.
- Develop a biomarker to detect stress in dolphins.
- Define methylation sites and quantify methylation in the promoter region of the serotonin transporter.
- Compare methylation of promoter regions from different individuals.

Materials & Methods

Blood

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DNA isolation using the Qiagen Blood & Tissue Kit

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Bisulfite conversion using the EZ DNA Methylation-Lightning Kit by Zymo Research

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PCR

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Quantitative methylation analysis through bisulfite pyrosequencing

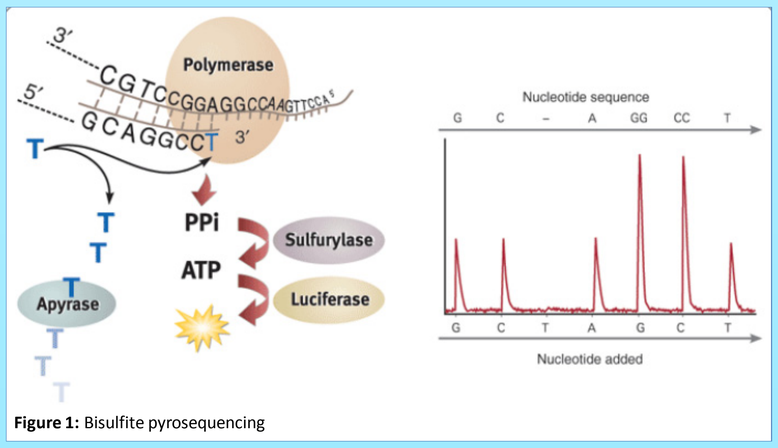

Figure 1: Bisulfite pyrosequencing

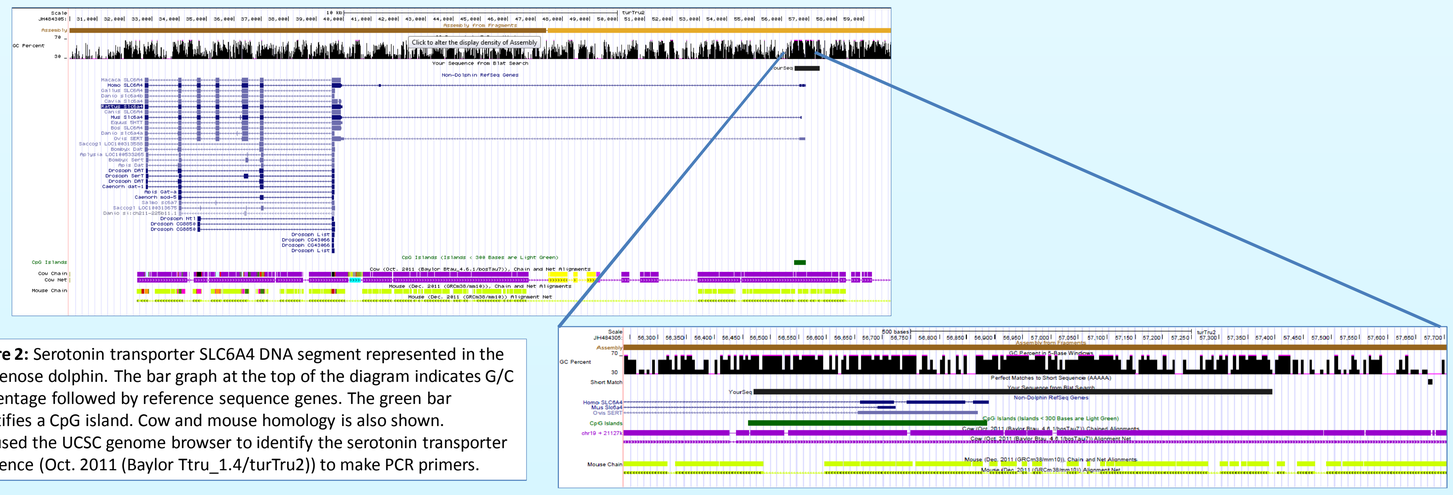

Figure 2: Serotonin transporter SLC6A4 DNA segment represented in the bottlenose dolphin. The bar graph at the top of the diagram indicates G/C percentage followed by reference sequence genes. The green bar identifies a CpG island. Cow and mouse homology is also shown. We used the UCSC genome browser to identify the serotonin transporter sequence (Oct. 2011 (Baylor Ttru_1.4/turTru2)) to make PCR primers.

Results

PCR Results

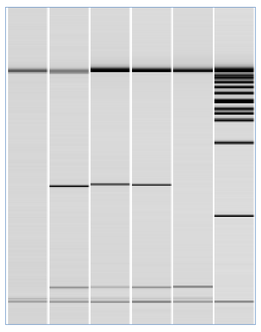

Figure 3: Electrophoresis of blood samples from dolphin 1 (lanes 1 & 2) and dolphin 2 (lanes 3 & 4). Water control in lane 5.

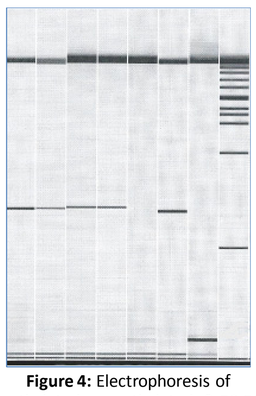

Figure 4: Electrophoresis of methylated, unmethylated, & 50:50 controls from dolphin 1 (lanes 1, 2, & 3) and from dolphin 2 (lanes 4, 5, & 6). Water control in lane 7.

Quantitative Methylation Analysis Through Bisulfite Pyrosequencing

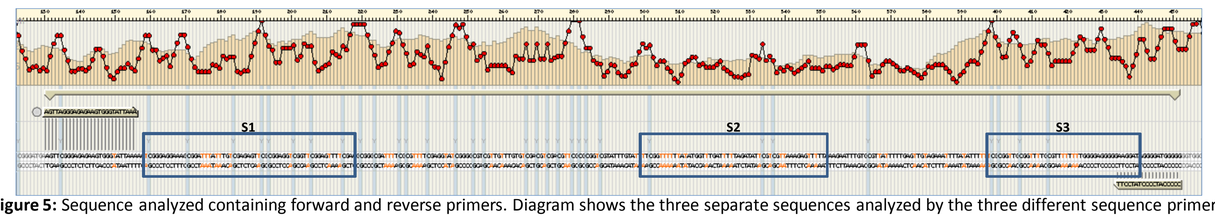

Figure 5: Sequence analyzed containing forward and reverse primers. Diagram shows the three separate sequences analyzed by the three different sequence primers.

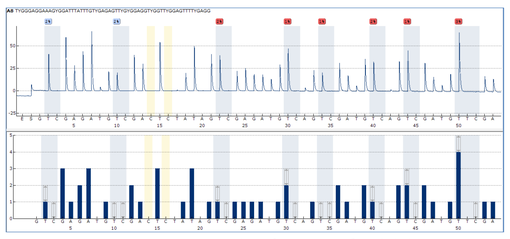

Figure 6: Sequence results of primer 1 for dolphin 2.

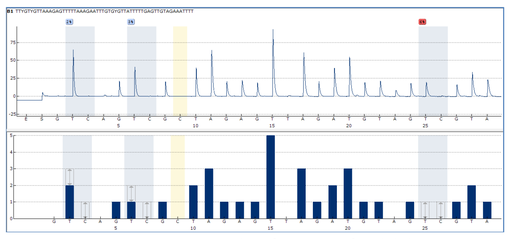

Figure 7: Sequence results of primer 2 for dolphin 2.

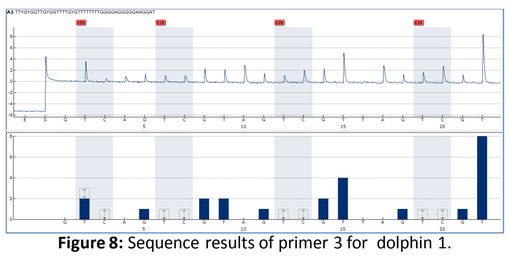

Figure 8: Sequence results of primer 3 for dolphin 1.

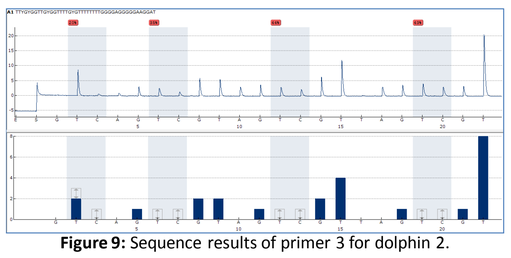

Figure 9: Sequence results of primer 3 for dolphin 2.

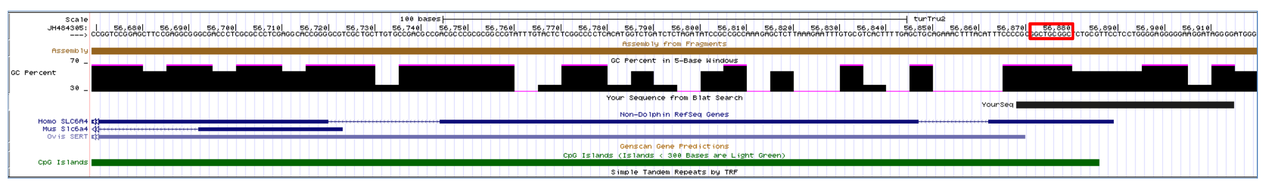

Figure 10: Region analyzed by sequence primers. The bar graph indicates the G/C percentage. Also shown are reference sequence genes along with CpG island information. No methylation was seen in the regions analyzed by primers 1 and 2 (only sequence analyzed by primer 2 and 3 are shown). The red box depicts an area of the promoter region that showed partial methylation detected by primer 3.

Conclusions

- We have identified three potential differentially methylated CpG sites within the SLC6A4 promoter region.
- Most sites investigated were unmethylated but three sites showed partial methylation that varied between the two individuals examined.
- We were able to detect methylation in the promoter region of the SLC6A4 gene (using sequence primer 3) while CpGs located within exon 1 (detected by sequence primers 1 and 2) showed no methylation.

Future Directions

- Determine if dolphins have a polymorphism in the SLC6A4 gene similar to that found in humans.
- Carry out an extensive study with tissues from multiple stranded animals, and tissues from non-stranded controls.
- Perform whole genome epigenetic analysis on several individuals.
- Investigate other candidate stress genes.

Acknowledgements

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