Investigating gene-environment interactions of methylmercury using zebrafish

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Introduction & Hypothesis

Methylmercury (MeHg) is an environmental pollutant found in the Great Lakes and a known danger to prenatal health. The upper limit considered to be safe is 5.8 ppb for newborns in a blood spot taken at birth. In the Lake Superior region, 1% of umbilical cord blood spots from have levels above 58 ppb (10x the safe level), and up to a maximum of 211 ppb (McCann, 2011). Children prenatally exposed to methylmercury can show a range of neurological deficits, from subtle developmental delays to cerebral palsy (Castoldi et al., 2001). There is thus a need for further understanding molecular and functional alterations due to methylmercury exposure. Knowing more about the alternations due to methylmercury exposure could help prevent neurological deficits in children prenatally exposed to methylmercury.

MeHg is eliminated as gluthaione (GSH) conjugates. Recent studies of human genetic polymorphisms have revealed that certain alleles of GSH-related genes − GSTP1, GCLC and GCLM are associated with elevated blood Hg levels in adults (Schläwicke Engström et al., 2008; Custodio et al., 2004). We are using a zebrafish model to study the influence of human polymorphisms on neuronal development following MeHg exposure. We have demonstrated that zebrafish exposed during development to low-to-moderate levels of MeHg show neurological deficits during development and as adults (Carvan Lab, UW-Milwaukee).

<u>Hypothesis:</u> Zebrafish lacking functional genes involved in MeHg metabolism that are exposed to MeHg in development have enhanced neurological deficits compared to wild-type zebrafish.

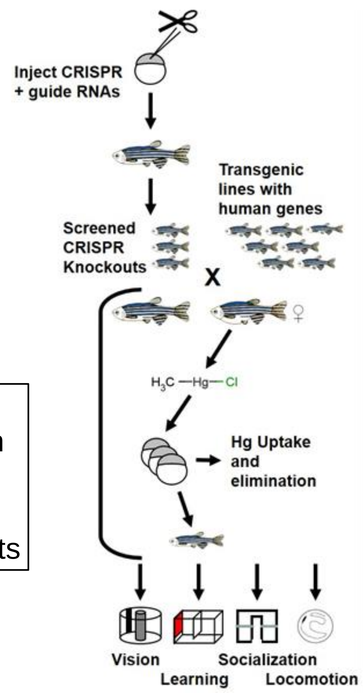

Figure 1: Experimental plan for CRISPR mutagenesis and testing MeHg effects

Methods; establishing CRISPR protocols

CRISPR protocol pipeline

Design gRNA
↓
Synthesize gRNA
↓
Breed zebrafish
↓
Collect embryos
↓
Microinject embryos; gRNA + Cas9 protein
↓
Screen for mutations ~2 days

Figure 2: Microinjector set up used for CRISPR injections

Figure 3: Designing gRNAs using CHOPCHOP online tool

Pair	Left primer coordinates	Left primer	Left primer Tm	Left primer off-targets	Right primer coordinates	Right primer	Right primer Tm	Right primer off-targets	Pair off-targets	Product size
1	seq:111-132	AGAAGAGTACGCTTTTTGGGG	59.8	1	seq:229-251	AGATGTTGAGGAACTGCAGGAT	60.1	1	0	140
2	seq:111-132	AGAAGAGTACGCTTTTTGGGG	59.8	1	seq:230-252	TAGATGTTGAGGAACTGCAGGA	59.9	1	0	141
3	seq:111-132	AGAAGAGTACGCTTTTTGGGG	59.8	1	seq:228-249	ATGTTGAGGAACTGCAGGATG	60.1	1	0	138
4	seq:84-106	ACGTGAAGTGTGCGTAAGTTGA	60.8	1	seq:211-232	GATGCCGTGTTTTCTTACGTG	60.5	1	0	148
5	seq:111-132	AGAAGAGTACGCTTTTTGGGG	59.8	1	seq:230-251	AGATGTTGAGGAACTGCAGGA	59.9	1	0	140

Off-targets

Figure 3: CHOPCHOP is an online tool used to select target sites for CRISPR mutagenesis and design associated genotyping primers (Laben et al 2019). CHOPCHOP was used in our experiment to select target sequences for our gRNAs for each gene we are knocking out (e.g. *tyr)*.

Figure 4: CRISPR targeting of pigment gene (*tyr)* results in mutation

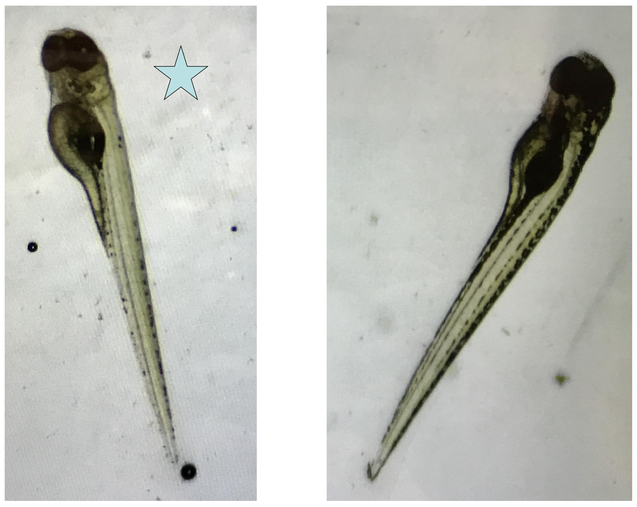

Figure 4 : Zebrafish on the left (star) is the tyrosinase mutant, showing less melanin pigmentation. The zebrafish on the left is a uninjected control zebrafish, showing the normal melanin pigmentation.

Figure 5: CRISPR reagents for mutagenesis of *tyr* show ~50% efficiency

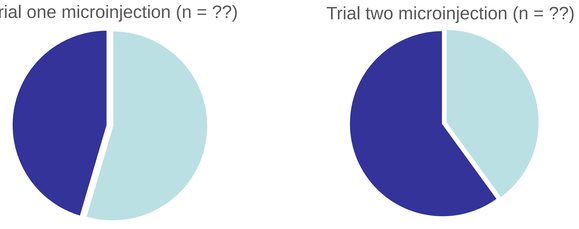

Figure 3: Ratios of mutants to non-mutants are shown in pie chart form from two separate CRISPR injection experiments.
Light-blue = non-mutants, dark-blue = mutants

Analysis and Interpretation

Injecting zebrafish embryos with *tyr* gRNA and Cas9 protein yielded tyrosinase mutants. An important part of this result was excluding glycerol from the Cas9. We had multiple failed trials when glycerol was included in the Cas9. The main success of this experiment was establishing a protocol for this study and any future zebrafish studies done at UWEC using CRISPR.

Future Directions

Long-term, we plan to use high-resolution melt analysis (HRMA) PCR to detect mutations created by CRISPR injections. We will first establish a protocol for HRMA PCR for confirming *tyr* mutagenesis. After establishing a protocol for HMRA PCR, we plan to design and implement CRISPR reagents for methylmercury metabolism genes.

<u>Acknowledgements</u>

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<u>CITATIONS</u>

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