Identification of the functional mechanism of alcohol-Wnt signaling pathway interactions in tooth development

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INTRODUCTION

Fetal Alcohol Spectrum Disorder (FASD)

- Birth defects associated with prenatal alcohol exposure
- Include general growth retardation, craniofacial defect, central nervous system abnormalities
- It is reported to be in 20%- 30% of live births
- Each year in Canada it is estimated that nine babies in every 1000 live births are born with FASD

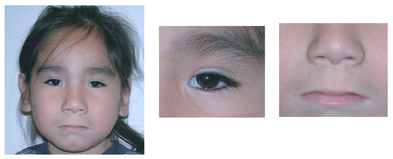


Figure 1: Features used to diagnose the fetal alcohol syndrome (FAS) facial phenotype are short palpebral fissures, a smooth philtrum, and a thin upper lip, as exhibited by this child

- Craniofacial and dental anomalies represent 80% of the defects observed in children with FASD
- Clinical and experimental animal studies show that embryonic alcohol exposure can affect the early Tooth Development (TD).

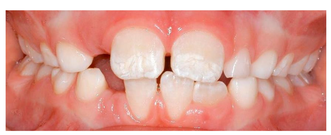


Figure 2: Enamel defects (hypo-mineralization) in FASD patient

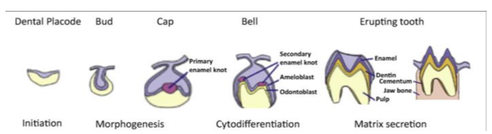


Figure 3: **Different stages during TD**

- The cell signaling pathway molecules such as Wnt signaling pathway are important for proper TD .

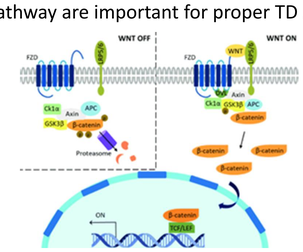
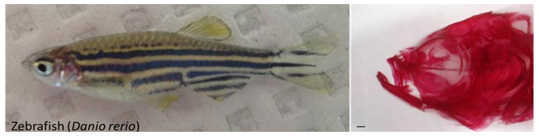


Figure 4: Overview of canonical Wnt signaling pathways

- Studies on Zebrafish (*Danio rerio*) provide many advantages toward understanding the morphogenetic mechanisms of craniofacial development, including TD.

Advantages of Zebrafish as a model animal

- Easy to study early development since they have external fertilization
- Amenable for molecular and genetic analysis
- Easy laboratory care/inexpensive
- Short life cycle and generation time
- Optically transparent embryos
- Availability of genetic knockdown tools



Dentition of Zebrafish

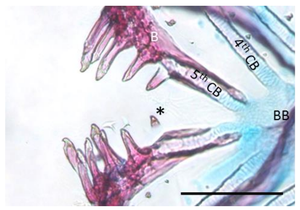


Figure 5: Pharyngeal dentition of 20dpf double-stained Zebrafish. asterisk shows an unattached tooth. Scale bar: 100 nm. Magnification: ×20. 4th CB, forth ceratobranchial. 5th CB, fifth ceratobranchial. BB, basibranchial. B, bone.

HYPOTHESIS

We hypothesized that interaction of alcohol-Wnt pathway during embryonic development can cause defects in TD.

METHOD

- A total of 350 Zebrafish embryos were treated with 1% alcohol (EtOH), Wnt pathway activator (2mM LiCl) and inhibitor (10nM WC59) at 10 hours post fertilization (hpf).
- Samples were fixed at 15, 20, 25 and 30 days post fertilization (dpf) to analyze the dental phenotypes.
- Whole mount cartilage and bone staining and ultrastructural microscopic analysis were carried out for examination of tooth number, length and width measurements, mineralization, and cusp shape.
- The independent T-test analysis were performed, and statistically significant outcomes reported as $P < 0.05$ in all analyses.

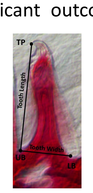


Figure 6: Microscopic measurements for checking morphological differences of the tooth

RESULTS

- The teeth of all treated samples were deformed evident from straight cusp morphology compared to hook-like cusp in control samples.
- No significant change was seen in the number of teeth in treated samples compared with controls ($P > 0.05$).
- Further, the length and width of teeth in the EtOH, EtOH combined with LiCl, WC59, and EtOH combined with WC59 treated samples were significantly less than the control ($P < 0.001$).
- However, the length and width of LiCl-treated samples were significantly higher than the control ($P < 0.001$).

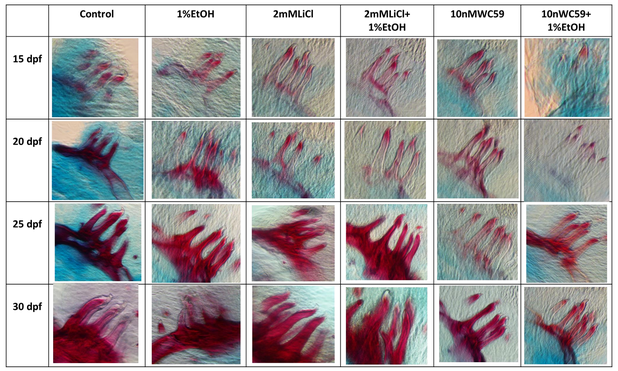
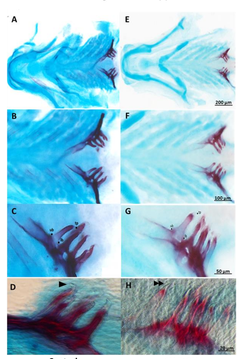


Figure 7: Acid-free double-stained tooth-bearing pharyngeal bones of Zebrafish. Control samples show unicuspid teeth which directly attached to the underlying bone. Samples exposed to 1% EtOH, 10nMWC59 and combine treatment of 1%EtOH + 2nMLiCL and 1%EtOH + 10nMWC59 at 10 h post-fertilisation show small, malformed and hypo-mineralised teeth. Samples exposed to 2mMLiCl show larger and hyper-mineralized teeth.



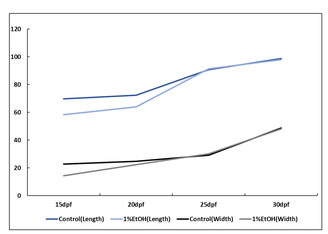


Figure 9: Comparing the tooth length and width in control and 1% EtOH treated samples

Figure 8: Acid-free double-stained tooth-bearing pharyngeal bones of Zebrafish at 15 days post-fertilisation (dpf). (A–D) Control samples that show tooth-bearing lower pharyngeal bones with six teeth in each bone. These teeth are unicuspid and directly attached to the underlying bone. At this stage the teeth are fully mineralized. **(E–H)** Samples exposed to 1% EtOH at 10 h post-fertilisation show small, malformed and hypo-mineralized teeth. lb: lower base; tp: tip of the tooth; ub: upper base. **(D and H)** Enlarged view of the pharyngeal teeth with hooked cusp in control (arrowhead) and straight cusp in 1% EtOH treated sample (double arrowhead)

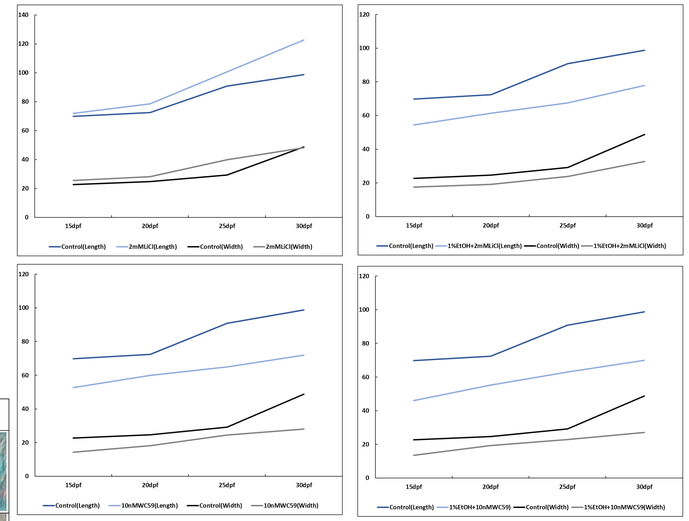


Figure 10: Comparing the tooth length and width in control and 2mMLiCl, 1% EtOH + 2mMLiCl, 10nMWC59, 1%EtOH + 10nMWC59 treated samples

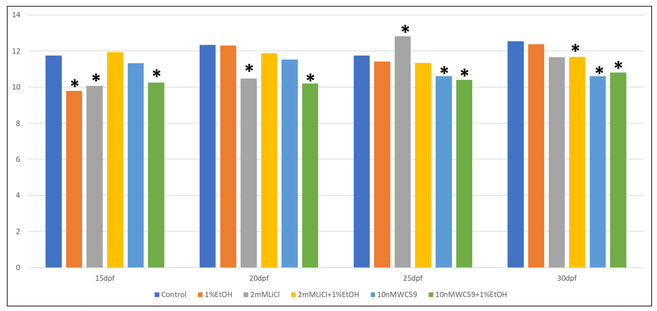


Figure 11: Comparing the tooth number in control and different chemical treated samples at 15, 20, 25 and 30 dpf. Asterisks show P<0.05.

CONCLUSION

The results support our hypothesis, and it is concluded that alcohol exposure and Wnt–alcohol interactions affect the development and patterning of the dentition in Zebrafish.

ACKNOWLEDGEMENTS

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The Children's Hospital Research Institute of Manitoba

CHRD CHILD HEALTH RESEARCH DAYS

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