Genetic Factors Underlying Susceptibility to Prenatal Alcohol and Cannabinoid Exposure

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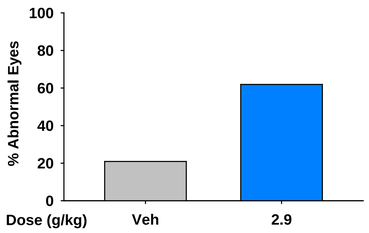
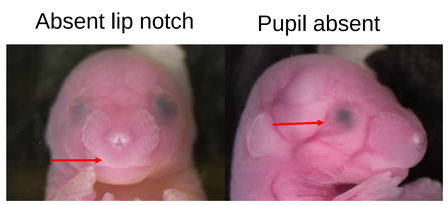
INTRODUCTION

- Prenatal alcohol and cannabinoid exposure cause birth defects.
- Both drugs inhibit Sonic hedgehog (Shh) signaling, which is involved in craniofacial development.
- C57BL/6J mouse strain is highly susceptible to developing craniofacial defects after prenatal alcohol and cannabinoid exposure. The 129S1/Svmlj strain's susceptibility is not well studied.
- Alcohol is a general upstream Shh pathway inhibitor.
- CP-55,940 (synthetic cannabinoid) is a Smo inhibitor and CB1 agonist.
- Vismodegib is a synthetic Smo inhibitor.

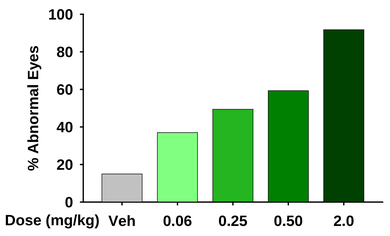
Background Data From C57BL/6J

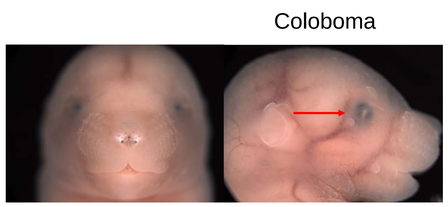
Prenatal Alcohol Exposure Causes Eye Defects



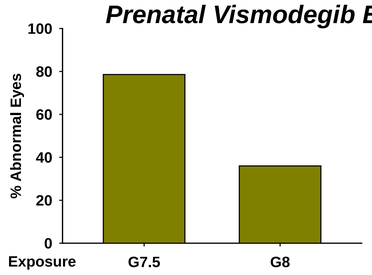


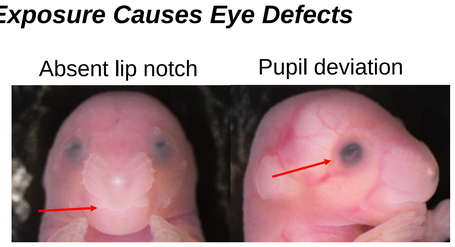
Prenatal Cannabinoid Exposure Causes Eye Defects





Prenatal Vismodegib Exposure Causes Eye Defects

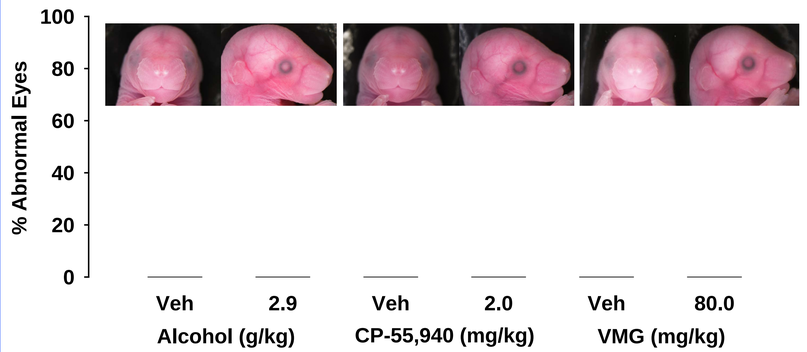




OBJECTIVES

- Determine 129S1 strain susceptibility to Shh antagonists.
- Determine transcriptomic differences between B6J and 129S1 strains accounting for susceptibility differences.

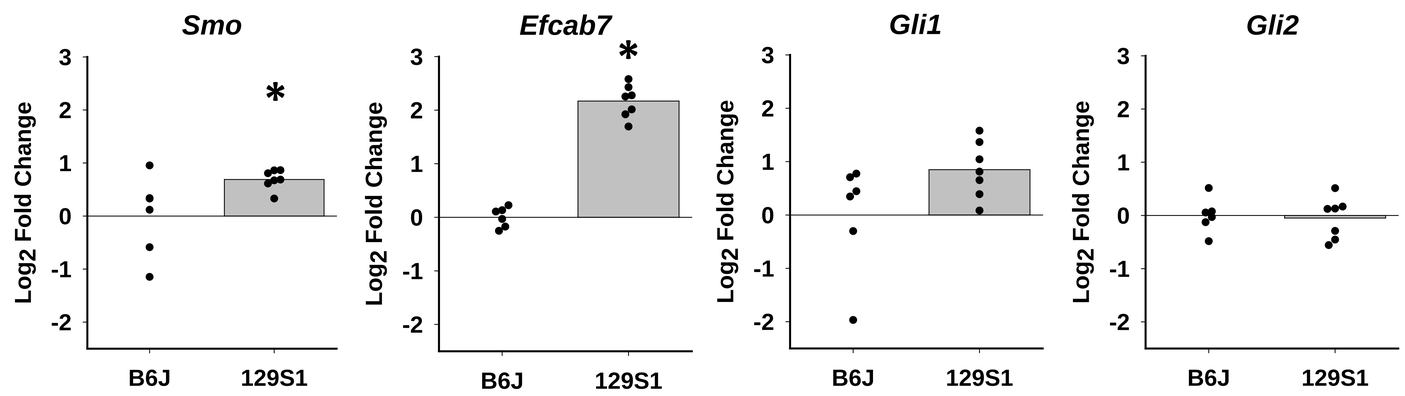
129S1/Svmlj Teratogenic Susceptibility



	Alcohol		CP-55,940		Vismodegib	
	Veh	2.9 g/kg	Veh	2.0 mg/kg	Veh	80 mg/kg
Litter Size	7.33 ±0.56	7.06 ±0.50	7.25 ±0.53	7.13 ±0.44	5.83 ±0.79	7.86 ±0.99
Resorptions	1.83 ±0.59	0.81 ±0.23	2.00 ±0.85	1.50 ±0.27	1.33 ±0.61	1.57 ±0.65
Weight	0.75 ±0.01	0.74 ±0.01	0.73 ±0.02	0.70 ±0.02	0.83 ±0.05	0.74 ±0.02
Length	1.71 ±0.01	1.73 ±0.01	1.67 ±0.02	1.65 ±0.01	1.76 ±0.05	1.70 ±0.04

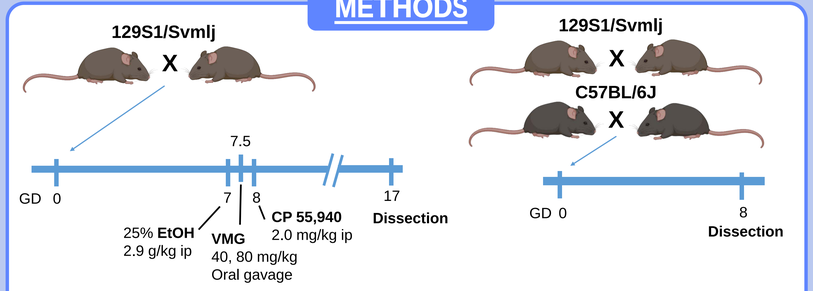
No 129S1 mice developed eye defects. There was not an observed effect of treatment on litter size, resorptions, weight, or length for any drug tested. 129S1 mice are insensitive to prenatal alcohol, cannabinoid, and vismodegib exposure. There were no significant differences between vehicle and treatment groups.

Strain Differences in Shh-Related Gene Regulation



Smo (p=0.04), *Efcab7* (p<0.0001), and *Gli1* (p=0.08) are upregulated in 129S1 embryos. *Gli2* is not different between strains (p=0.79). **The Shh pathway is upregulated in 129S1 mice, so they have a greater capacity to resist Smo inhibition due to the larger relative expression of *Smo*.**

METHODS



Morphological Assessment

- Body weight
- Crown-Rump Length
- Craniofacial and Eye Defects

qRT-PCR

- Whole embryo
- RNA isolation and dilution
- RNA → cDNA
- Reference gene: *Rpn1*

SUMMARY & CONCLUSIONS

- The 129S1 strain is not susceptible to birth defects from exposure to three Shh pathway antagonists, two of which are specific Smo inhibitors.
- Shh-related genes *Smo* and *Efcab7* were significantly upregulated in 129S1 strain, indicating a possible explanation for 129S1 resistance to teratogenesis.
- We are in the process of performing RNA-sequencing to expand our understanding of transcriptomic differences between strains.