Retinoic Acid Deficiency: An Etiology of FASD

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Background: Prenatal alcohol exposure (PAE) resulting in Fetal Alcohol Spectrum Disorder (FASD) is the most common cause of neurodevelopmental impairments in the western world, with a prevalence of 1-2% in Canada. It is well established in *Xenopus* models that a single exposure to alcohol during gastrulation is sufficient to induce the developmental defects associated with FASD.

Hypothesis: Acute ethanol exposure overwhelms the aldehyde metabolic enzymes that would normally convert retinol (Vitamin A) to retinoic acid (RA). We hypothesize that PAE reduces RA levels during critical developmental stages in early gastrulation and this aberration drives the later craniofacial malformations associated with FASD.

Methods: To biochemically mimic the alcohol-induced RA deficiency *in vivo,* we genetically engineered a mouse expressing Cyp26A1 from the endogenous *Goosecoid* (*Gsc*) promoter. The *Gsc* promoter dictates spatial-temporal expression to the Spemann Mangold Organizer at the start of gastrulation. Cyp26A1 degrades endogenous RA in these cells, mimicking the reduced RA levels induced by acute alcohol exposure and dysregulating the induction of neural crest cells.

Results: *Gsc:Cyp26A1* mice were derived by germline transmission; F1 mice are born with a Mendelian ratio of 0.66:1 (het:wt , n=351). This loss of mutant embryo viability may reflect the prevalent miscarriages observed in human pregnancies with PAE. *Gsc:Cyp26A1*x*RARE-LacZ* E8.5 embryos show reduction in RA activity in the frontonasal prominence region (LacZ expression in early face and forebrain). Mutant embryos also demonstrate body-axis developmental variation, indicating early developmental perturbation of retinoic acid pathways in FASD relevant tissues (n=48). E18.5 embryos were next examined using scanning electron microscopy to demonstrate mutant embryos have sentinel FASD craniofacial malformations: larger philtrum-to-philtrum-lip length ratio, smaller bigonial line width, and smaller whisker pad area compared to WT littermates (n=66). *Gsc:Cyp26A1* mice also develop craniofacial malocclusions at significantly higher rates than WT littermates (12.5% vs 0.04%; n=208 and 3711, respectively).

Conclusion: Taken together, our data provide *in vivo* evidence that strongly supports retinoic acid deficiency as a major molecular etiology of craniofacial malformations associated with FASD. The finding suggests Vitamin A supplementation may significantly reduce or prevent FASD outcomes in children with PAE.
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