Risk and Resilience Variants in the Retinoic Acid Network and Developmental Pathways Influence FASD Outcomes

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Introduction

Fetal Alcohol Spectrum Disorder (FASD) is the most common neurodevelopmental disorder in children, with a prevalence of 1-5% in Canada[1]. Unfortunately, a majority of those with FASD remain undiagnosed and this suggests the prevalence of FASD is much higher than estimated. There is no treatment or cure for FASD, nor is the etiology of the disorder fully known. Alcohol dosage[2,3], duration of exposure and gestational timing[4-6], such as during key developmental processes like gastrulation, are all important determinants of the induction and severity of FASD outcomes following prenatal alcohol exposure (PAE). Other risk factors, such as maternal genetics, epigenetics, nutrition, metabolism, and stress[7–11] each to some degree contribute towards the severity of FASD outcomes as well. Studies in both animal models and humans have shown that aside from maternal alcohol metabolizing genes[12,13], the genetics of the embryo itself also determines PAE outcomes[14-19]. However, our knowledge of the genetics of FASD is limited and further study is needed to discover which genes underlie FASD outcomes. The vitamin A hypothesis of FASD postulates that ethanol's teratogenic outcomes are largely due to its inhibition of the conversion of retinol (vitamin A) to its active signaling form, retinoic acid (RA), during the development of the embryo (Figure 1). RA upon binding to its receptors, functions as a essential regulator of embryonic development, guiding neural crest proliferation, migration and differentiation. Ethanol's inhibitory effects on RA synthesis and consequent perturbation of neural crest cell development give rise to the FASD sentinel features (Figure 2). Given ethanol's inhibitory effect on RA synthesis during development, it is likely that variants in the RA network, which includes known PAE susceptibility genes, will influence FASD outcomes in humans.

Hypothesis: We hypothesize that variants in retinoic acid metabolism and retinoic acid-dependent developmental signalling pathways genetically link acute PAE with FASD outcomes.

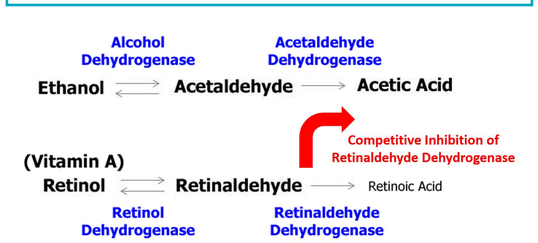

Figure 1. Vitamin A Hypothesis of Fetal Alcohol Spectrum Disorder. Illustration depicting the shared familial enzymes between the Alcohol (Ethanol) and Vitamin A (Retinol) pathways. Upon acute alcohol exposure the enzyme normally converting retinaldehyde to retinoic acid (RALDH 2) is instead usurped to convert acetaldehyde to acetic acid, resulting in a reduction in retinoic acid synthesis and retinoic acid deficiency.

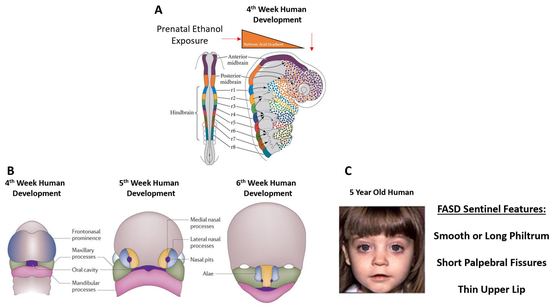

Figure 2. Perturbation of Neural Crest Cell Development by Ethanol-Induced Retinoic Acid Deficiency Give Rise to the FASD Sentinel Features. Signalling through a concentration gradient throughout the embryo, retinoic acid when bound to its receptors, functions as a transcription factor to guide neural crest cell development. (panel A) The retinoic acid gradient guides cranial neural crest cell migration from the hindbrain into the developing craniofacial area, giving rise to both the face and the forebrain. Prenatal ethanol exposure results in retinoic acid deficiency and disrupts its signaling gradient, consequently perturbating neural crest cell migration into the craniofacial area (panel B). Following the retinoic acid gradient cranial neural crest cells from the hindbrain migrate into the frontonasal prominence give rise to the maxillary and mandibular processes, which ultimately allow the formation of the craniofacial area and consequently development of the face and forebrain. The FASD sentinel features include a smooth or long philtrum (lack of a vertical groove between the base of the nose and the border of the upper lip), short palpebral fissures (small eye openings) and a thin upper lip. These features are a result of prenatal ethanol induced retinoic acid deficiency and improper neural crest migration into the frontonasal prominence and maxillary processes (panel C). (Adapted from Dixon et al., 2011 and Wattendorf et al., 2005)

Methods

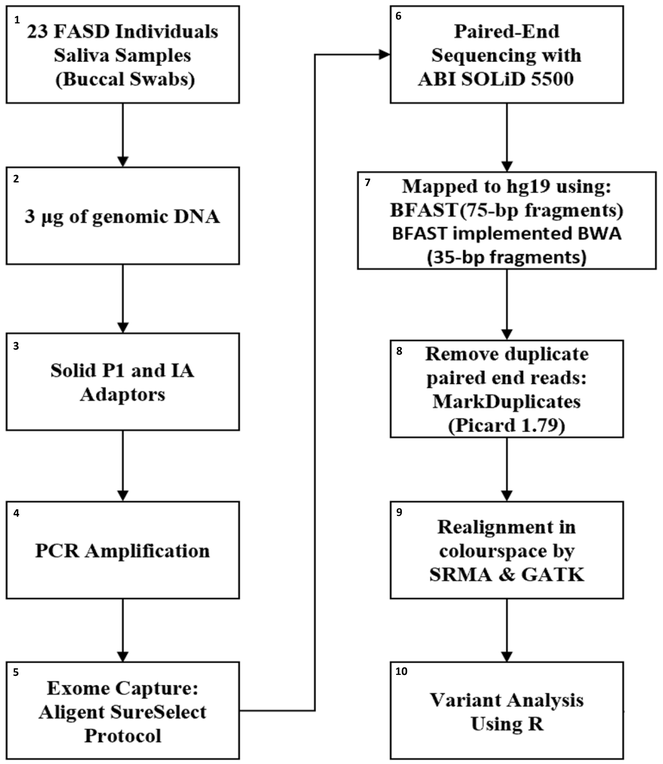

Figure 3. Methodology Employed to Complete Variant Analysis of FASD Cohort. Buccal swab samples were obtained from 23 FASD diagnosed individuals from FASD clinics across Canada. The ages of the patients ranged from 5 to 18, half were female and the other half were male. Around half of the cohort was of European ancestry, while the remaining half was of First Nations, Inuit or Metis ancestry. We also had 3 pairs of sibling in the cohort. DNA was extracted from the acquired samples and sent off for whole exome sequencing at the Center of Applied Genomics located at The Hospital for Sick Children in Toronto Canada, who processed the data through steps 3 to 9. R was used to complete the variant analysis on the received whole exome sequencing data from the Center of Applied Genomics by comparing the allelic frequencies to control populations downloaded from Thousand Genomes. We employed a gene candidate approach for the variant analysis for our cohort. Our gene list of interest composed allelic variants found in retinoic acid metabolism and retinoic acid-dependent developmental signalling pathways.

Results

Table 1: Variant Analysis Results Alcohol and Retinoic Acid Metabolism Candidate Genes Sensitizing Variants

rsID	Gene	Type of Mutation	Candidate Gene List	Risk Allele	Biological Implication of Variant	FASD Risk Allele Frequency	Thousand Genomes Risk Allele Frequency	p-value
rs971074	*ADH7*	Synonymous	Alcohol Metabolism	T	Risk allele associated with higher drug and alcohol dependence	10/46 (0.22)	1200/10016 (0.12)	0.028
rs1229984	*ADH1B*	Missense	Alcohol Metabolism	C	Risk allele associated with more alcohol consumption, alt allele is more kinetically active (more alcohol oxidation)	45/46 (0.97)	8428/10016 (0.84)	0.017
rs698	*ADH1C*	Missense	Alcohol Metabolism	C	Risk allele is associated with more alcohol consumption, ref allele 1.5 to 2x kinetically faster	25/46 (0.55)	2146/10016 (0.22)	2.7E-7
rs2073478	*ALDH1B1*	Missense	Alcohol Metabolism	T	Risk allele associated with alcohol dependence	27/46 (0.59)	2146/10016 (0.39)	0.03
rs971756	*STRA6*	Missense	Retinoic Acid Metabolism	T	Risk allele associated with Mathew-Woods Syndrome	6/46 (0.13)	546/10016 (0.05)	0.002
rs11857410	*STRA6*	Missense	Retinoic Acid Metabolism	A	Risk allele associated with anophthalmia/microphthalmia	6/46 (0.13)	618/10016 (0.06)	0.005
rs2134095	*RXRG*	Synonymous	Retinoic Acid Metabolism	A	Risk allele associated with elevated LDL-cholesterol levels and gestational diabetes mellitus	34/46 (0.74)	4796/10016 (0.48)	0.002
rs35361223	*BCO2*	Missense	Retinoic Acid Metabolism	G	Risk allele predicted to be deleterious to protein function	7/46 (0.15)	184/10016 (0.02)	1.13E-15

Table 2: Variant Analysis Results Retinoic Acid Developmental Network Candidate Genes Resilience Variants

rsID	Gene	Type of Mutation	Candidate Gene List	Risk Allele	Biological Implication of Variant	FASD Cohort Risk Allele Frequency	Thousand Genomes Risk Allele Frequency	p-value
rs1044006	*NOTCH3*	Synonymous	Retinoic Acid Developmental Network	C	Risk allele associated with mandibular prognathism	33/46 (0.72)	8732/10016 (0.87)	2.3E-3
rs35415678	*AXIN2*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele could disrupt exonic splicing enhancer sequences and contribute to tooth agenesis	1/46 (0.02)	1646/10016 (0.16)	0.0147
rs1802074	*SFRP4*	Missense	Retinoic Acid Developmental Network	T	Risk allele associated with failing hearts; ref allele associated with increased SFRP4 expression	4/46 (0.09)	2534/10016 (0.25)	0.0254
rs228226	*GLI1*	Missense	Retinoic Acid Developmental Network	C	Risk allele associated with congenital heart defects and hypospadias	7/39 (0.15)	5872/10016 (0.32)	0.027
rs2230808	*ABCA1*	Missense	Retinoic Acid Developmental Network	C	Risk allele associated with Smith-Lemli-Opitz syndrome and lower levels of cholesterol	11/46 (0.24)	4624/10016 (0.46)	0.0104
rs2066836	*PTCH1*	Synonymous	Retinoic Acid Developmental Network	G	Risk allele associated with cleft lip and palate	31/46 (0.67)	9096/10016 (0.91)	4.8E-5

Table 3: Variant Analysis Results Retinoic Acid Developmental Network Candidate Genes Sensitizing Variants

rsID	Gene	Type of Mutation	Candidate Gene List	Risk Allele	Biological Implication of Variant	FASD Cohort Risk Allele Frequency	Thousand Genomes Risk Allele Frequency	p-value
rs1044006	*NOTCH3*	Synonymous	Retinoic Acid Developmental Network	C	Risk allele associated with mandibular prognathism	0.72	0.87	2.3E-3
rs1049007	*BMP2*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele associated with ossification defects in posterior longitudinal ligament	0.74	0.25	1.7E-3
rs235768	*BMP2*	Missense	Retinoic Acid Developmental Network	T	Heterozygous genotype (A/T) associated with cleft lip and palate	0.57	0.77	3.8E-3
rs17563	*BMP3*	Missense	Retinoic Acid Developmental Network	C	Risk allele associated with tooth defects and cleft lip and palate	0.5	0.33	0.0368
rs113683	*AXIN2*	Synonymous	Retinoic Acid Developmental Network	A	AA (alt allele) genotype associated with cleft lip and palate	0.83	0.45	6.1E-8
rs2908004	*WNT16*	Missense	Retinoic Acid Developmental Network	G	Risk allele increases canonical WNT signalling and decrease non-canonical WNT signalling, and associated with bone mineral density and fracture risk	0.67	0.49	0.045
rs2707466	*WNT16*	Missense	Retinoic Acid Developmental Network	C	Risk allele increases canonical WNT signalling and decrease non-canonical WNT signalling, and associated with bone mineral density and fracture risk	0.7	0.49	0.027
rs12549394	*FZD6*	Missense	Retinoic Acid Developmental Network	A	Risk allele associated with neural tube defects	0.39	0.2	4.01E-3
rs1047057	*FGF2*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele associated with Apert's Syndrome (Het genotype)	0.65	0.59	0.049
rs35415678	*AXIN2*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele could disrupt exonic splicing enhancer sequences and contribute to tooth agenesis	0.09	0.23	0.047
rs1802074	*SFRP4*	Missense	Retinoic Acid Developmental Network	T	Risk allele associated with failing hearts; ref allele associated with increased SFRP4 expression	0.09	0.25	0.254
rs4819756	*PRODH2*	Missense	Retinoic Acid Developmental Network	A	Risk allele noted to drop catalytic activity of enzyme by 30-70% and associated with schizophrenia	0.54	0.22	5.2E-7
rs11542682	*KRIT*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele associated with cerebral cavernous malformations, epilepsy, headache, cerebral hemorrhages and focal neurological deficits	0.96	0.82	0.0271
rs41298840	*TBX1*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele associated with conotruncal heart defects in children with 22q11.2DS	0.913	0.769	0.46
rs139884	*SOX10*	Synonymous	Retinoic Acid Developmental Network	G	Het genotype discovered in a patient of Waardenburg Syndrome Type I	21/46 (0.46)	7144/10016 (0.71)	5.1E-4
rs1051886	*WNT10B*	Synonymous	Retinoic Acid Developmental Network	A	Risk allele associated with hip bone mineral density	24/46 (0.52)	3116/10016 (0.31)	7.5E-3
rs2240308	*AXIN2*	Missense	Retinoic Acid Developmental Network	A	Risk allele associated with frontal agenesis, oral cleft, congenital heart and atrial septal defects	30/46 (0.65)	3382 (0.34)	3.64E-5
rs7984	*RHO*	3'UTR	Retinoic Acid Developmental Network	A	Risk allele associated with age-related macular degeneration and retinitis pigmentosa	37/46 (0.804)	4724/10016 (0.47)	3.83E-5

Discussion and Significance

Overall, variant analysis revealed enrichment of PAE-sensitizing variants, and a lack of protective variants for the FASD cohort compared to the reference population. The identification of risk alleles underlying a PAE-induced RA deficiency during development provides a new molecular etiology driving FASD outcomes, including neural tube, brain and craniofacial defects. These results identify potential new predictive biomarkers and novel therapeutic targets for prevention or reduction of FASD outcomes following acute PAE.

Future Directions

Molecular Inversion Probes (MIP) assays will be completed in order to validate the presence of statistically significant genetic variants in our samples[20] (see Figure 3 for overall workflow pipeline of the first cohort). Following variant validation, Enrichr will be used to determine which genetic and biological pathways are upregulated and downregulated in our cohort based on statistically-significant genes[21]. We are also awaiting the genetic sequencing data of two more FASD cohorts, one composed of 100 children and the second of 36 twin pairs from the Manitoba FASD clinic, which will processed in a similar manner to the 23 individual FASD WES cohort and used to validate our initial findings.

Acknowledgements

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