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Alcohol Intubation and Synergistic Effect of Folic Acid and Egg Yolk Powder Solution on Alcohol Dehydrogenase [ADH] Class I Gene Expression and Single Nucleotide Polymorphism Frequency in Parent and Prenatal Alcohol Exposed Wistar Rats

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Abstract: Point mutation detections in alcohol metabolizing genes could unravel molecular targets of gestational alcohol and epigenetic remodeling capacity of maternal nutrition, which underscores growth and health endpoints in Prenatal Alcohol Exposed (PAE) fetuses. gene expression was assayed using sequenced target and internal control gene primers in a two-step thermal cycling process from isolated RNA in purely bred alcohol male and female Norway rats weighing between 200-220 g. They were mated and grouped as follows: (A) Distilled water only [negative control)., (B) Local gin only, (C) Local gin supplemented with egg yolk solution, (D) Local gin supplemented with egg yolk solution and folic acid (E) EtOH only (positive control). Equal doses of 3.0 mL/kg/bw were administered before, during, and after gestation via oral gavage. ADHI gene subtypes in the pups of mated rats were analyzed by the methods used in the parental stocks. The PCR products were electrophoresed on a 2.5% agarose gel while bioinformatics databases for SNP detection were used for the sequenced data analyses in the alcohol-dosed parent and PAE rats. a total number of 35,838 bp coding DNA of the ADH1 gene were sequenced in the experimental rats with 115 random synonymous and non-synonymous SNP distributions. Parent-of-origin inheritance pattern of ADHI was confirmed in PAE neonates while SNPs frequency in sequence data of ADHI subtypes in groups' B-E dicts functional alcohol effects and changes in resultant protein. Hence, this study provides evidence for paternal and maternal contributions and nutrient-gene interactions in PAE rats.

Keywords: Alcohol, Gene Expression, Prenatal, Gestational, Epigenetic, Genomic Imprinting

Introduction

Variation in metabolism rate, compulsive alcohol use, altered behavior, and deleterious effects has been attributed to Single Nucleotide Polymorphisms (SNPs) occurrence in alcohol metabolizing genes in PAE fetuses and alcohol users (Ong *et al*., 2018; Katsarou *et al*., 2017). Non-synonymous SNPs (nsSNPs) comprise a group of SNPs that, together with SNPs in regulatory regions, are believed to have the highest impact on phenotype (Abid *et al*., 2023; Robert and Pelletier, 2018). Based on gene expression and sequence alignment, there are seven distinct genes relating to the five enzyme classes namely: Class 1-ADH1A (MIM %103700), ADH1B and ADH1C; class II, ADH4 (MIM % 103740); class III, ADH5 (MIM %103710); class IV, ADH7 (MIM %600086); and class V, ADH6 (MIM %103735) (Osier *et al*., 2002). Some polymorphic

variants in enzymes involved in alcohol metabolism (parental and fetal genotypes) have been closely linked to faster blood alcohol ethanol clearance and subsequent alcohol abuse. Differential expression of the ADH class I gene haplotypes (ADH1A, ADH1B, and ADH1C) confirms associations of genetic polymorphism, giving credence to differing levels of alcohol intoxication and metabolism effects in individuals within and between diverse populations (Chien *et al*., 2023). Unbalanced dosage of the maternal and paternal genomes also provides a controlled biological system for studying the effects of allelic dosage and parental effects on gene expression, as some genes are preferentially expressed (Barlow and Bartolomei, 2014) and could either be erased during reprogramming (Daxinger and Whitelaw, 2012) or stably maintained all through adult life. There are events where the expression of a gene into a trait is influenced by the allelic parent of origin as well as by the activity state of the locus in the parent's genome (Macias-Velasco *et al*., 2022). The parental origin of genetic material has an impact on gene expression and this effect is known as genomic imprinting. There is evidence of genomic imprinting effects on traits, behavior, and the etiology of certain neurobehavioral disorders (Isles, 2022), which include fetal alcohol syndrome. Imprinting occurs when the expression of a gene is influenced by maternal or paternal transmission such that one allele is preferentially expressed (Tucci *et al*., 2019). Genomic imprinting is influenced by differential DNA methylation during gametogenesis (Paro *et al*., 2021), as specific genes become active at particular times during development. Increased global DNA methylation has been reported in alcoholic patients when compared with normal controls (Tulisiak *et al*., 2017) and this implies the possibility of silenced genes in alcoholics. The investigation of possible epigenetic mechanisms in addition to genetic studies has been proffered as a means to better understand complex observable characteristics that occur as a result of environmental signals and exposures (e.g., drugs, diet, aging, etc.,) during development in living systems (Tiffon, 2018). This is because epigenetic interactions are thought to mediate alcohol's adverse effect on fetuses. Maternal nutrition during each of the trimesters could also cause epigenetic changes that could potentially induce physiological changes that result in disease or protection from disease (Peral-Sanchez *et al*., 2021; Barchetta *et al*., 2021) and have been proven to worsen disease severity or outcome. Associations between PAE outcomes and alcohol metabolizing gene polymorphisms as well as nutrient-gene interactions are critical for a proper understanding of the molecular targets of alcohol during pregnancy. Hence, we aimed to investigate the impact of alcohol on metabolizing genes (ADH1) as well as possible nutrient-gene interactions as a result of egg yolk powder and folic acid intubation in the experimental rats used in this study.

Materials and Methods

The experimental animals used in this study were sourced from the Anatomy Department of Afe Babalola University in Ado Ekiti. The rats were housed in adequately ventilated cages and given time to acclimate to the controlled environmental conditions of the experimental facility. These conditions were maintained at a temperature range of 28-31°C, with a natural light-dark cycle of 12 h each and a humidity level between 50 and 55%.

Chemicals used in the study included absolute ethanol (C_2H_5OH, Sigma Aldrich^{TM}), with a molecular weight of 46.07 and a boiling range of 77-79°C. Folic acid tablets (Vitabiotics^{TM}) weighing 20 mg each were also employed, along with 40% concentrated local gin (brand name withheld). The absolute ethanol was diluted to a concentration of 40% using all-glass laboratory-distilled water for use as synthetic alcohol. Egg yolk powder was derived from freshly laid chicken eggs and utilized in the experimentation process.

Experimental Rats Mating Based on ADH1 Allelic Class and Dose Regimen

Female alcohol naïve purely bred rats of *norvegicus* strain weighing between 200-220 g were grouped into five (A) and mated with a male rat in duplicate groups (1-2). Yolks from freshly laid eggs were freeze-dried using the method described by Wei *et al.* (2019) for a 0.5 g/mL dietary egg yolk solution preparation. A 3.0 mL/kg/bw equal dose regimen was delivered in the following groups: (A) Distilled water (negative control), (B) Local Gin (LG), (C) Local Gin + Egg yolk solution (LGE) (D) Local gin + egg yolk solution folic acid LGEF and (E) 40% EtOH (positive control group). Alcohol doses were diluted before administration to reduce gastric and esophageal irritation and all doses were administered via oral gavage with food and water given and libitum. Ethical clearance for the study was sought and assigned the approval number: UERC/ASN/2016/604. After gestation and weaning of pups (postnatal day 30), rats and pups were sacrificed and their liver was severed for immediate RNA isolation.

RNA Isolation and cDNA Synthesis

RNA isolation and characterization were carried out for a total of 30 samples from rats' hepatic tissues. Total RNA isolation was done using Zymo Quick RNA miniprep kits and quantified with 1µg of template RNA in Nanodrop 2.0 for concentration and purity values (Fig. 1). First-strand cDNA synthesis of samples was carried out using ProtoScript II first-strand cDNA and synthesized using laboratory established amplification protocols at cycles 94-56.5°C for 30 sec and 94°C for 1 min in Bio-Rad 1000 thermal cycler.

Table 1: Sequenced primers for target gene (ADH1) characterization and internal control

Gene	Primer	Product size (bp)
ADH1	F: ATGAGCACAGCTGGAAAAGTA	1081
	R: TCAGAACGTCAGGACGGTACG	
GAPDH	F: ACCACAGTCCATGCCATCAC	461
	R: TCCACCACCCTGTTGCTGTA	

PCR Amplification

The second step Polymerase Chain Reaction (PCR) amplification was performed in a final volume of 40 µL using One Taq Quickload 2× Master mix at 25°C for 5 min, 42°C for 60 min, and 80°C for 5 min. Synthesized cDNA from pups, weaned mother rats, and father rats were used to assay for their respective ADH1 subtypes using primers flanking each of the exon sequences of ADH1 and GAPDH (internal control) respectively. The nucleotide blast (BLASTn) query was used for ADH1 allelic characterization in parental stocks and their respective pups' sequence data. Sequenced primers for ADH1 and GAPDH are stated in Table 1.

Agarose Gel Electrophoresis of PCR Amplicons and Sequencing

The PCR products were electrophoresed on a 2.5% agarose gel and the 1081 bp ADH1gene sequence data for the Norway rat was retrieved from the NCBI database while the expected fragment size for the target gene and internal control were determined using the Amplify software. Purification, sequencing, and quantification of PCR products were done in the ABI 3500× L genetic analyzer using standard sequencing parameters (sanger sequencing). Sequenced data was analyzed for *ADH*I classification using bioinformatics software and NCBI database submissions (ADHI) classes and frequency shown in (Fig. 1).

SNPs Description and Amino Acid Change

Defined open reading frames (ORFs) and reference sequences were displayed from available databases. Mutation and SNP detection through base substitution was assayed via further alignment with DNABaser™ which defined the amino acid changes in the codons (Table 2).

Results

ADHI Subtypes Distribution in Rats

In parental stock rats, allelic gene frequency of ADH, ADH1A, ADH1C was highest (50%), 35% score was recorded for ADH1A while the least expressed allelic subtypes were ADH-like protein (10%) and ADH mRNA and introns (5%) (Fig. 1 and Table 2).

Alcohol Effects on Male and Female Rats' RNA Concentration/Yield

RNA yield was significantly higher in male rats than female rats in the following groups (Fig. 2): A (Distilled water Σ46.20/32.80 ng/µL, $p<0.05$), C (LGE) (Σ88.40/51.60 ng/µL, $p<0.001$) while a statistically insignificant difference was obtained in local gin (group B) and LGEF (group D) rats (Σ126.2/121.7, Σ97.70/95.30 ng/µL, $p>0.05$).

Highest RNA concentrations were observed in weaned pups from groups B (LG)- Σ200.10 ng/µL $p<0.0001$, C (LGE) -Σ95.10 ng/µL $p<0.001$ and E (EtOH) -Σ88.50 ng/µL $p<0.01$ while there was no significant difference in data obtained from pups of negative control (A) and LGEF (D) rats (Σ36.1/34.4 ng/µL, $p>0.05$ respectively) (Fig. 2).

Nucleotide BLAST query launch of sequenced data from pups (Table 2) revealed exclusive expression of either maternal or paternal ADH1 allelic gene frequency as follows: (a) ADH, ADH1A, ADH1C (50%), (b) ADH1A (30%), (c) ADH like protein (10%) and (d) ADH mRNA and introns (10%).

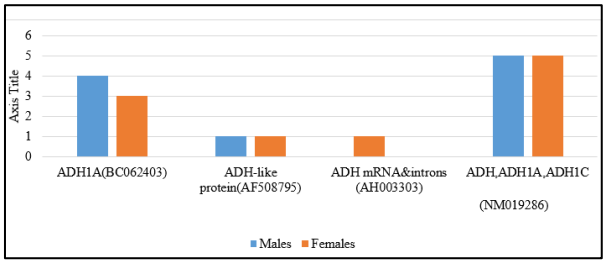

Fig. 1: ADH1 subtypes distribution in parent rats and frequency with NCBI ascension numbers

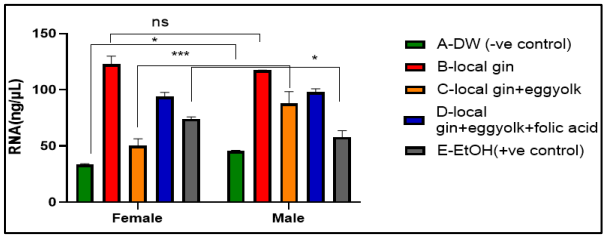

Fig. 2: Alcohol effect on male and female parent rats' hepatic RNA concentration (N = 4, * = $p<0.05$, *** = $p<0.001$)

Table 2: ADH1 subtypes heritability, SNPS and genetic codon changes in alcohol-dosed parents and pups

SN	Groups	Retrieved accession number	NCBI bp/ rats' ADH1 base pair	ADH1 subtypes in P1	Parental allele heritability	No of SNPs	Position of SNP	Point mutation	fS/fN	Base changes
1	A1 male	NM019286	1806/1065	ADH, ADH1A, ADH1C		1	34	A-T	1/0	ATC-TTC
2	A1 female	NM019286	1806/1801	ADH, ADH1A, ADH1C		2	32, 740	C-G, T-A	2/0	TCC-TGC, ATC-AAC
3	A1 pup	NM019286	1806/1336	ADH, ADH1A, ADH1C	Maternal/Paternal	1	117	T-A	1/0	CCT-CCA
4	A2 male	NM019286	1806/1050	ADH, ADH1A, ADH1C		2	55, 88	A-T, A-T	2/0	AAG-TAG, AAA-TAA
5	A2 female	BC062403	1336/1336	ADHIA		2	32, 116	G-C, C-G	2/0	TGC-TCC, TTC-TTG
6	A2 pup	BC062403	1336/895	ADHIA	Maternal	1	611	A-T	1/0	AGT-TGT
7	B1 male	NM019286	1806/1061	ADH, ADH1A, ADH1C		2	991, 1068	C-A, T-G	0/2	CTG-ATG, GCT-GCG
8	B1 female	BC062403	1336/1062	ADHIA		1	75	A-T	1/0	TCA-TCT
9	B1 pup		1806/1066	ADH, ADH1A, ADH1C	Paternal	1	1049	C-A	0/1	GCC-GAC
10	B2 male	BC062403	1336/1031	ADHIA		2	31, 119	T-A, C-T	1/1	TGC-AGC, CCA-CTA
11	B2 female	NM019286	1806/1070	ADH, ADH1A, ADH1C		19	50, 51, 63, 67, 71, 80, 84,119,347, 470, 794, 951, 968, 1033, 1039, 1047, 1049, 1050, 1054	C-R, A-R, C-R, A-S, C-W, A-S, C-T, A-R, C-Y A-S, A-R, C-, Y, G-T, A-W, G-C, G-A, T-A, A-T, A-T, C-A, C-T, C-A	8/11	ACA-ARA, ARA-ARR, AGC-AGR, AGC-SGC, ACA-ATA, AAA-ARA, GTA-GTS, CAC-CRC, TCT-TYT, GGA-GTA, AAC-AWC, GTG-GTC, AGC-AAC, TTT-ATT, AGT-TGT, GAA-GAT, GCC-GAC, GCC-GCT, CCC-CCA
12	B2 pup	NM019286	1806/1336	ADH, ADH1A, ADH1C	Maternal	4	121, 156, 178, 879	A-M, C-Y, A-W, C-G	2/2	AAG-MAG.CCC-CCY, ATT-WTT, GGC-GGG
13	C1 male	BC062403	1336/1070	ADHIA		2	66, 69	T-C, G-Y	1/1	GAA-GAT. AAG-AAY
14	C1 female	NM019286	1806/552	ADH, ADH1A, ADH1C		5	81, 122, 350, 473, 797	A-K, A-Y, C-S, T-R, C-Y	2/3	AGT-KGT, AAG-AYG, CCC-CSC, AAG-ARG, CCT-CYT
15	C1 pup	BC062403	1336/1336	ADHIA	Paternal	4	26, 29, 31, 348	T-W, C-W, T-M, A-S	1/3	ATT-AWT, TCC-TWC, TGC-MGC, GAA-GAS
16	C2 male	NM019286	1806/1061	ADH, ADH1A, ADH1C		2	793, 991	A-G, C-T	0/2	AAC-GAC, CTG-TGT
17	C2 female	BC062403	1336/1068	ADHIA		2	30, 347	C-A, A-T	1/1	TCC-TCA, GAA-GTA
18	C2 pup	BC062403	1336/1289	ADHIA	Maternal	4	32, 33, 36, 112	G-A, C-T, A-C, C-G	1/3	TGC-TAC, CCC-TCC, CCA-CCCq1t, CCC-CGC
19	D1 male	NM019286	1806/1063	ADH, ADH1A, ADH1C		10	80, 951, 968, 1033 1047, 1049, 1050, 1054,1058,1066	A-G, G-A, G-C, A-T, T-C, C-G, C-G, C-G, A-C, G-C	6/4	AAA-AGA, GTG-GTA, AGC-ACC, AAA-TTT, GAT-GAC, GCC-GGC, GCC-GCG, CCC-GCC, AAA-ACA, GCT-CCT
20	D1 female	AF508795	1365/1051	ADH like protein		3	55, 80.127	G-C, G-A, C-G	2/1	GGA-CGA, AGT-AAT, CAC-GAC
21	D1 pup	NM019286	1806/1060	ADH, ADH1A, ADH1C	Paternal	3	25, 37, 43	C-G, C-G, C-T	2/1	CGA-GGA, CAT-GAT, CTG-TTG
22	D2 male	NM019286	1806/1061	ADH, ADH1A, ADH1C		2	32, 740	C-M, T-Y	0/2	TCC-TMC, ATC-AYC
23	D2 female	NM019286	1806/1068	ADH, ADH1A, ADH1C		6	59, 61, 69, 70, 90, 96	A-T, C-W, C-W, A-Y, A-W, T-M	2/4	GAC-GTC, AGC-WGC, AGC-AGW, ACA-YCA, AAA-AAW, AAT-AAA
24	D2 pup	NM019286	1806/1489	ADH, ADH1A, ADH1C	Maternal/Paternal	2	35, 60	T-A, C-G	2/0	ATC-AAC, GAC-GAG
25	E1 male	BC062403	1336/521	ADHIA		2	190, 194	G-A, C-T	0/2	CGG-AGG, CGA-TGA
26	E1 female	AH003303	3657/3657	ADH mRNA and introns		18	25, 30, 66, 88.94, 109, 118, 347, 945, 949, 970, 972, 993, 1004, 1011, 1012, 1046, 1047	G-W, G-M, C-T, T-A, C-W, G-W, T-Y, C-S, N-S, N-W, N-Y N-Y, A-R, A-K, A -S, C-S, C-W, A-W	10/8	GTG-WTG, AAG-AAM, AGC-AGT, TTG-ATG, CAG-WAG, GGG-WGG, TCT-YCT, CCT-CST, NNN-NNS, NNN-NWN, NNN-YNN, NNN-NNY, GAAGAR, TAA-TKA, ACA-SCA, ACA-SSA, TCA-TWA, TWA-TWW
27	E1 pup	AH003303	3657/981	ADH mRNA and introns	Maternal	4	26, 29.31, 348	T-W, A-W, G-M, T-S	4/0	GTG-GWG, GAA-GAW, GGC-MGC, CCT-CCS
28	E2 male	BC062403	1336/1065	ADHIA		2	30, 347	C-M, A-C	1/1	TCC-TCA, GGA-CCA
29	E2 female	AF508795	1365/1071	ADH like protein		3	34, 36, 84, 1043	C-W, A-M, T-K, G-W	1/3	CGT- WGT, CCA-CCM, AAT-AAK, AGA-AWA
30	E2 pup	AF508795	1365/1060	ADH like protein	Maternal	2	34, 112	C-A, G-C	1/1	GGT-AGC, GGA-GGA

SNP Changes and Frameshift Genetic Codon Changes in Alcohol-Dosed Parents and Pups

Although synonymous SNPs were observed in negative controls and alcohol groups, the highest nonsynonymous SNP changes were observed in the local gin (B1 and B2) female parents with functional base changes in the genetic codons as analyzed using the mutation detection software (Table 2).

Discussion

Molecular characterization of sequenced data from alcohol-dosed and prenatal alcohol-exposed rats in nucleotide Basic Local Alignment Search program (BLASTN) revealed four ADH1 subclasses in the parental stock which are: (a) ADH1A; (b) ADH, ADH1A, ADH1C; (c) ADH like protein and (d) ADH mRNA and introns. The high degree of variability in the RNA yield in alcohol dose groups (supplemented and non-supplemented Figs. 2-3) compared to negative control rats could be due to transcription factors induced by alcohol intubation (Wallén *et al*., 2021; Miles, 1995) resulting in the possibility of altered cellular function and genic function. House-keeping gene for endogenous alcohol dehydrogenase gene expression studies (GAPDH) bands were present in all Agarose Gel Electrophoresis (AGE) images (Fig. 4) of PCR products of all rats in the study (regardless of dose group) as GAPDH is critical for basic cellular and metabolic functions and also maintains stable and constitutive expression (Eisenberg and Levanon, 2013). This suggests alcohol intubation, folic acid, and egg yolk solution did not impact disruptive effects on rats' ADH1

constitutive expression. Several alcohol researches on candidate gene expression investigations of alcohol effects on promoters indicated DNA methylation as an important biomarker of heavy alcohol consumption (Yousefi *et al*., 2019; Liu *et al*., 2018) and is inadvertently linked to SNP frequencies in gene data (De Souza *et al*., 2020) in this study. SNPs occurrence in the non-coding regions of negative control ADH subtypes is similar to Teixeira *et al*. (2015) finding of SNPs as a naturally occurring event in the LAD1 gene of Bonobos and Chimpanzees. However, the frequency of first and second nucleotide base and nsSNPs changes in alcohol-only groups suggest epigenetic remodeling effects of alcohol which was minimal in the supplemented groups. Active substitutions that have deleterious downstream effects could destabilize interactions between nucleic acid networks and bonding. Also, Zhou *et al*. (2013) identification of sequence differences in gene coding regions between alcohol-preferring and non-alcohol-preferring rats by exome sequencing indicates possible functional effects of alcohol metabolizing genes on metabolizing enzymes, proteins, and alcohol preference. Reduced SNPs frequency in supplemented groups compared with non-supplemented alcohol groups in this study suggests folic acid and egg yolk supplementation effects in bioavailability of choline which is chronically reduced in alcohol-fed rats due which can be attributed to alcohol metabolites effects on maternal nutrition profile impairments (Steane *et al*., 2021; Thomas *et al*., 2010). There was also a distinct expression of either maternal or paternal ADH1 inheritance in the F_1 rats. The distinct maternal or paternal allelic expression of the ADH1 gene (non-additive gene expression) observed in the alcohol-exposed rats filial one rats confirms evidence of ADH1 as an imprinted gene i.e., expression is determined by the contributing parent. Imprinted genes do not conform to the usual rule of inheritance that both alleles in a heterozygote are equally expressed (Onyango *et al*., 2002), but a form of epigenetic inheritance whereby the regulation of a gene or chromosomal region is dependent on the sex of the transmitting parent (MacDonald, 2012).

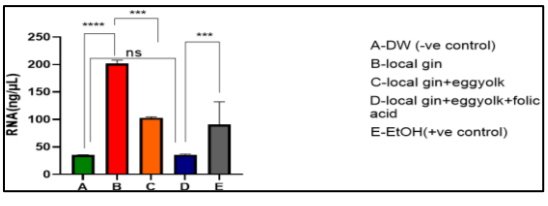

Fig. 3: Prenatal alcohol effect on pups' hepatic RNA concentration (N = 4, * = p<0.05, *** = p<0.001)

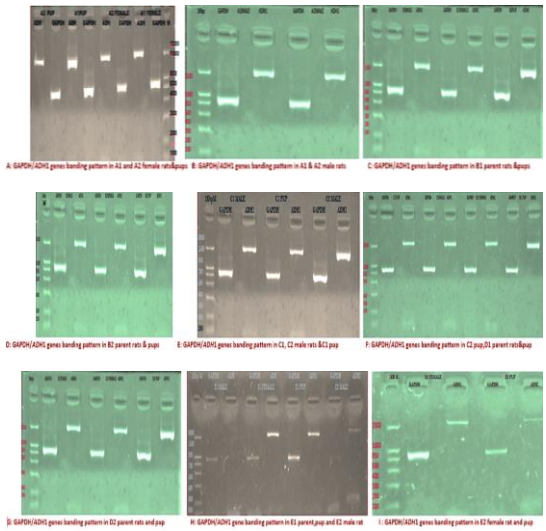

Fig. 4: Agarose gel electrophoresis of housekeeping gene (GAPDH) and ADH1 gene in parent and pup rats

Conclusion

Nucleotide base changes of rats used in this study revealed SNPs as a spontaneous event and housekeeping gene expression profiles in AGE indicate ADH1 gene constitutive expression regardless of alcohol intubation and nutrient supplementation. However, the frequency of non-synonymous to synonymous base changes in non-supplemented and supplemented alcohol groups confirms the epigenome remodeling capacity of alcohol while nutrient-gene interactions of egg yolk solution and folic acid supplementation can be explored for modifying outcomes in PAE fetuses. Also, PAE rats in this study revealed high levels of non-additive allelic expression of ADH1 gene thereby establishing non-additive expression and parent-of-origin pattern of ADH1 gene inheritance in same.

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Author's Contributions

Adejoke Olukayode Obajuluwa: Conception, study designed, execution, acquisition of data, analysis, interpretation and manuscript drafted.

Oluyinka Ajibola Iyiola and Joseph Akintade Morakinyo: Conception and study designed.

Tiwalola Madoc Obajuluwa: Execution, acquisition of data and study designed.

Rahman Ayodele Bolarinwa and Adetunji Adesina: Execution, acquisition of data and interpretation.

Ethics

Ethical clearance for the study was obtained and assigned the approval number: UERC/ASN/2016/604.

Conflicts of Interest

The authors of the manuscript declare no conflict of interest concerning the work.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

References

Abid, F., Khan, K., Badshah, Y., Ashraf, N. M., Shabbir, M., Hamid, A., ... & Razak, S. (2023). Non-synonymous SNPs varia+nts of PRKCG and its association with oncogenes predispose to hepatocellular carcinoma. *Cancer Cell International*, *23*(1), 1-24. https://doi.org/10.1186/s12935-023-02965-z

Barchetta, I., Arvastsson, J., Sarmiento, L., & Cilio, C. M. (2021). Epigenetic changes induced by maternal factors during fetal life: Implication for type 1 diabetes. *Genes*, *12*(6), 887. https://doi.org/10.3390/genes12060887

Barlow, D. P., & Bartolomei, M. S. (2014). Genomic imprinting in mammals. *Cold Spring Harbor Perspectives in Biology*, *6*(2), a018382. https://doi.org/10.1101/cshperspect.a018382

Chien, T. H., Lin, C. L., Chen, L. W., Chien, C. H., & Hu, C. C. (2023). Patients with Non-Alcoholic Fatty Liver Disease and Alcohol Dehydrogenase 1B/Aldehyde Dehydrogenase 2 Mutant Gene Have Higher Values of Serum Alanine Transaminase. *Journal of Personalized Medicine*, *13*(5), 758. https://doi.org/10.3390/jpm13050758

Daxinger, L., & Whitelaw, E. (2012). Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nature Reviews Genetics*, *13*(3), 153-162. https://doi.org 10.1038/nrg3188

De Souza, A. M., Lopes, O. S., de Lima Liberato, A., de Oliveira, P. J. R., Herrero, S. S. T., do Nascimento, A. L., ... & Lima, E. M. (2020). Association between SNPs and loss of methylation site on the CpG island of the promoter region of the smoothened gene, potential molecular markers for susceptibility to the development of basal cell carcinoma in the Brazilian population. *Asian Pacific Journal of Cancer Prevention: APJCP*, *21*(1), 25. PubMed: 31983159.

Eisenberg, E., & Levanon, E. Y. (2013). Human housekeeping genes, revisited. *TRENDS in Genetics*, *29*(10), 569-574. https://doi.org/10.1016/j.tig.2013.05.010

Isles, A. R. (2022). The contribution of imprinted genes to neurodevelopmental and neuropsychiatric disorders. *Translational Psychiatry*, *12*(1), 210. https://doi.org/10.1038/s41398-022-01972-4

Katsarou, M. S., Karakonstantis, K., Demertzis, N., Vourakis, E., Skarpathioti, A., Nosyrev, A. E., ... & Drakoulis, N. (2017). Effect of single-nucleotide polymorphisms in ADH 1B, ADH 4, ADH 1C, OPRM 1, DRD 2, BDNF and ALDH 2 genes on alcohol dependence in a Caucasian population. *Pharmacology Research and Perspectives*, *5*(4), e00326. https://doi.org/10.1002/prp2.326

Liu, C., Marioni, R. E., Hedman, Å. K., Pfeiffer, L., Tsai, P. C., Reynolds, L. M., ... & Levy, D. (2018). A DNA methylation biomarker of alcohol consumption. *Molecular Psychiatry*, *23*(2), 422-433. https://doi.org/10.1038/mp.2016.192

MacDonald, W. A. (2012). Epigenetic mechanisms of genomic imprinting: Common themes in the regulation of imprinted regions in mammals, plants and insects. *Genetics Research International*, *2012*. https://doi.org/10.1155/2012/585024

Macias-Velasco, J. F., St Pierre, C. L., Wayhart, J. P., Yin, L., Spears, L., Miranda, M. A., ... & Lawson, H. A. (2022). Parent-of-origin effects propagate through networks to shape metabolic traits. *Elife*, *11*, e72989. 11: e72989. https://doi.org/10.7554/eLife.72989

Miles, M. F. (1995). Alcohol's effects on gene expression. *Alcohol Health and Research World*, *19*(3), 237. PMID: 31798101.

Ong, H. H., Khor, F. V., Balasupramaniam, K., & Say, Y. H. (2018). Genetic polymorphisms of alcohol-metabolizing enzymes and their association with alcoholism risk, personality and anthropometric traits among Malaysian university students. *Psychology, Health and Medicine*, *23*(2), 160-170. https://doi.org/10.1080/13548506.2017.1338737

Onyango, P., Jiang, S., Uejima, H., Shamblott, M. J., Gearhart, J. D., Cui, H., & Feinberg, A. P. (2002). Monoallelic expression and methylation of imprinted genes in human and mouse embryonic germ cell lineages. *Proceedings of the National Academy of Sciences*, *99*(16), 10599-10604. https://doi.org/10.1073/pnas.152327599

Osier, M. V., Pakstis, A. J., Soodyall, H., Comas, D., Goldman, D., Odunsi, A., ... & Kidd, K. K. (2002). A global perspective on genetic variation at the ADH genes reveals unusual patterns of linkage disequilibrium and diversity. *The American Journal of Human Genetics*, *71*(1), 84-99. https://doi.org/10.1086/341290

Paro, R., Grossniklaus, U., Santoro, R., Wutz, A., Paro, R., Grossniklaus, U., ... & Wutz, A. (2021). Genomic Imprinting. *Introduction to Epigenetics*, 91-115. https://doi.org/10.1007/978-3-030-68670-3_5

Peral-Sanchez, I., Hojeij, B., Ojeda, D. A., Steegers-Theunissen, R. P., & Willaime-Morawek, S. (2021). Epigenetics in the uterine environment: How maternal diet and ART may influence the epigenome in the offspring with long-term health consequences. *Genes*, *13*(1), 31. https://doi.org/10.3390/genes13010031

Robert, F., & Pelletier, J. (2018). Exploring the impact of single-nucleotide polymorphisms on translation. *Frontiers in Genetics*, *9*, 507. https://doi.org/10.3389/fgene.2018.00507

Steane, S. E., Fielding, A. M., Kent, N. L. andersen, I., Browne, D. J., Tejo, E. N., ... & Akison, L. K. (2021). Maternal choline supplementation in a rat model of periconceptional alcohol exposure: Impacts on the fetus and placenta. *Alcoholism: Clinical and Experimental Research*, *45*(10), 2130-2146. https://doi.org/10.1111/acer.14685

Teixeira, J. C., De Filippo, C., Weihmann, A., Meneu, J. R., Racimo, F., Dannemann, M., Nickel B, Fischer A, Halbwax M, Andre C, Atencia A, Meyer M, Parra G, Paabo S, Andres A. M (2015). Long-term balancing selection in LAD1 maintains a missense trans-species polymorphism in humans, chimpanzees and bonobos. *Molecular Biology and Evolution*, *32*(5), 1186-1196. https://doi.org/10.1093/molbev/msv007

Thomas, J. D., Idrus, N. M., Monk, B. R., & Dominguez, H. D. (2010). Prenatal choline supplementation mitigates behavioral alterations associated with prenatal alcohol exposure in rats. Birth Defects Research Part A: *Clinical and Molecular Teratology*, *88*(10), 827-837. https://doi.org/10.1002/bdra.20713

Tiffon, C. (2018). The impact of nutrition and environmental epigenetics on human health and disease. *International Journal of Molecular Sciences*, *19*(11), 3425. https://doi.org/10.3390/ijms19113425

Tucci, V., Isles, A. R., Kelsey, G., Ferguson-Smith, A. C., Bartolomei, M. S., Benvenisty, N., ... & Wilkins, J. (2019). Genomic imprinting and physiological processes in mammals. *Cell*, *176*(5), 952-965. https://doi.org/10.1016/j.cell.2019.01.043

Tulisiak, C. T., Harris, R. A., & Ponomarev, I. (2017). DNA modifications in models of alcohol use disorders. *Alcohol*, *60*, 19-30. https://doi.org/10.1016/j.alcohol.2016.11.004

Wallén, E., Auvinen, P., & Kaminen-Ahola, N. (2021). The effects of early prenatal alcohol exposure on epigenome and embryonic development. *Genes*, *12*(7), 1095. https://doi.org/10.3390/genes12071095

Wei, L. J., Alkarkhi, A. F., & Huda, N. (2019). Physicochemical properties of egg yolk powder from eggs of different types of bird. *International Journal on Advanced Science, Engineering and Information Technology*, *9*(1), 373. https://doi.org/10.18517/ijaseit.9.1.3046

Yousefi, P. D., Richmond, R., Langdon, R., Ness, A., Liu, C., Levy, D., ... & Zuccolo, L. (2019). Validation and characterisation of a DNA methylation alcohol biomarker across the life course. *Clinical Epigenetics*, *11*, 1-12. https://doi.org/10.1186/s13148-019-0753-7

Zhou, Z., Karlsson, C., Liang, T., Xiong, W., Kimura, M., Tapocik, J. D., ... & Goldman, D. (2013). Loss of metabotropic glutamate receptor 2 escalates alcohol consumption. *Proceedings of the National Academy of Sciences*, *110*(42), 16963-16968. https://doi.org/10.1111/acer.13776