Research Square

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Genome-wide DNA methylation analysis of the liver in adult guinea pigs born preterm and term highlights the importance Cry1 and circadian rhythm in males

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Abstract

Background: Preterm birth is associated with increased lifetime risk of cardiometabolic and neurodevelopmental disease, yet the underlying organ-specific epigenetic mechanisms remain poorly understood. While altered DNA methylation profiles have been reported in accessible tissues such as cord blood and placenta, the long-term epigenetic consequences within metabolically active organs remain largely unexplored

Results: We performed DNA methylation profiling (reduced representation bisulphite sequencing) in adult (9-month) livers from guinea pigs born preterm (gestational age, 62 days) versus term (gestational age 69 days) using an established clinically relevant precocial model. In males we identified 743 significant differentially methylated (5mC) CpGs and 24 significant differentially methylated regions (DMRs) between term born control and preterm born cases. These DMRs were enriched in genes involved in key metabolic and regulatory pathways including circadian rhythm, lipid metabolism, ferroptosis, autophagy, mitochondrial function, vascular signalling, immune regulation, and chromatin modification.

Notably, *CRY1* emerged as a potential convergent mechanistic node, exhibiting increased DNA methylation and reduced mRNA expression in preterm-born animals, consistent with epigenetic repression of gene regulation. This observation aligns with the established role of *CRY1*in circadian regulation and hepatic gluconeogenesis, suggesting disruption of circadian-metabolic integration following pre-term birth. Additional DMRs mapped to genes governing lipid oxidation (*PPARA*), ferroptosis and lipid peroxidation control (*GPX4*), autophagy/mitochondrial quality control (*UBXN6, VPS13D*), and chromatin repression programs (*KDM2B*), and were enriched for genes linked to metabolic and inflammatory phenotypes associated with later life phenotypes of preterm birth. These observations suggest that durable epigenetic reprogramming of hepatic metabolic networks, potentially increase susceptibility to later-life metabolic disease.

Conclusions: Preterm birth in males is associated with persistent, organ-specific DNA methylation alterations in the adult liver that converge across interconnected pathways governing circadian control, lipid homeostasis, mitochondrial quality control, vascular–immune signalling, and chromatin regulation. These findings provide the first evidence of long-term hepatic epigenetic programming following preterm birth and identify *CRY1*as a potential mechanistic link between early-life adversity and later-life metabolic disease risk. This work establishes a foundation for future multi-omic and cell-type–resolved studies aimed at identifying targets to mitigate long-term health consequences of preterm birth.

Background

Early-life environmental exposures can exert lasting effects on health and disease risk across the lifespan of an organism. Epigenetic mechanisms, DNA methylation (5mC), are prime candidates for mediating such programming effects. They provide a dynamic interface between genome and environment, orchestrating cell lineage specification and long-term gene regulation, and enabling an organism to dynamically respond to changes in environmental stimuli and “reset” gene expression accordingly [1,2]. Emerging evidence links preterm birth to altered methylation profiles in cord blood, placenta and buccal cells [3–9]. These studies demonstrate that epigenetic signatures of prematurity are detectable early in development, but human studies are necessarily restricted to tissues that can be sampled using minimally invasive techniques. Preterm birth is, however, a multi-system insult. The limitations of current clinical research techniques mean that it is not possible to directly interrogate metabolically active organs such as the liver; consequently, the molecular basis of hepatic epigenetic programming following preterm birth remains poorly defined. Despite these limitations, longitudinal human studies suggest that epigenetic differences following preterm birth may persist into adolescence and adulthood. For example, Kuula et al [10] reported distinct methylation signatures in young adults born very preterm. Similarly, reviews provide support for the role of epigenetic mechanisms in the developmental origins of health and disease, emphasising their potential to mediate long-term risk trajectories [11,12]. However, longitudinal investigation of the epigenetic changes consequent on preterm birth in human populations are restricted to peripheral blood measures, which provide limited insight into organ-specific programming.

To date, no study has characterised genome-wide methylation in metabolically active tissue following preterm birth, leaving a critical gap in understanding organ-specific epigenetic programming. Our study addresses this gap by directly examining the methylome of adult liver, a central metabolic organ and a major target of developmental programming, in a controlled animal model of preterm birth. This approach enables mechanistic interrogation of epigenetic remodelling in a tissue that is functionally relevant to the metabolic complications observed in humans born preterm.

The guinea pig (*Cavia porcellus*) offers a unique clinically relevant, translational model for addressing this gap. Unlike altricial rodents, guinea pigs exhibit precocial development, with *in utero* organ maturation and placentation patterns that closely resemble those of humans [13]. These features, combined with practical advantages of a small animal model, make the guinea pig an ideal system for investigating epigenetic consequences of preterm birth on organ development and function across the life course. As a result, guinea pigs are increasingly recognised as a powerful model for translating disease hypotheses, including the developmental origins of disease, to the clinic [13]. Guinea pig models of long-term survival following preterm birth have been developed in the last 13 years [13]. We previously described a novel

method of induction of preterm labour in guinea pigs, which resulted in viable pups and used comparable support to that provided to moderately preterm human infants [14]; this model has subsequently been used to study the long-term effects of preterm birth [15–20].

Here, we hypothesised that preterm birth leaves a persistent epigenetic imprint on the adult liver methylome. Our findings, in males, reveal widespread methylation differences enriched at metabolic regulatory elements, consistent with developmental programming of hepatic function. These findings have implications for understanding how early-life adversity shapes epigenetic architecture in key metabolic organs, informing strategies to mitigate long-term disease risk.

Methods

Experimental Model

The guinea pig model of preterm birth has been described in detail previously [14–20]. Briefly, confirmed time-mated Dunkin-Hartley sows were randomly allocated to term or preterm delivery using minimisation for maternal age and estimated litter size. Term pregnancies proceeded without intervention and delivered spontaneously at ~ 69 d gestation. Preterm birth was induced as described previously [14], using antenatal corticosteroids (betamethasone; 1 mg/kg sc; Celestone chronodose; Merck, Sharp & Dohme, Auckland, NZ) to mimic clinical management of threatened preterm labor, and progesterone receptor antagonism (aglepristone; 10 mg/kg; Provet, Palmerston North, NZ) and oxytocin (3 IU/kg i.m.; Provet, Palmerston North, NZ) to induce labour at 62 d gestation.

Neonatal stabilisation and postnatal care followed established methods [14,16]. Preterm pups remained with their mothers and received intensive neonatal care with respiratory, thermal and supplemental feeding until term-equivalent age (their term ‘due date’ equivalent to postnatal age (PNA) day 7). Following this they were housed with their mothers in standard conditions until weaning at corrected postnatal age (CPNA) 21d. All preterm-born pups and dams from their term equivalent age and all term-born pups and dams from birth were maintained in nursery pens under controlled temperature (18–23°C), humidity (50%), and a 12-hour light/dark cycle, with environmental enrichment. Animals had ad libitum access to guinea pig chow (Specialty Feeds, Australia), hay, fresh vegetables, and water supplemented with ascorbic acid (1.2 g/L). Following weaning at CPNA 21d, all offspring were housed in same-sex pens and maintained using the same environmental conditions and feeding regime.

At adulthood (CPNA 9m), animals were anesthetised and humanely killed by intracardiac injection of pentobarbital (1 mL; Provet). Death was confirmed by absence of heartbeat prior to tissue collection.

Samples

At 9 months CPNA liver samples were removed from euthanised animals and immediately snap frozen in liquid nitrogen and stored at -80C until use.

Liver samples from 12 adult male guinea pigs (6 born at term, 6 born preterm) were used for both DNA methylation and mRNA expression analyses. For additional gene expression analyses, RNA was extracted from liver samples from 11 female guinea pigs (6 born at term, 5 born preterm).

Nucleic Acid extraction

DNA and RNA were extracted from the same sample of snap frozen liver (~ 10mg) using the Qiagen AllPrep kit as per the manufacturers instructionsand the manufacturer modification for "Total RNA containing small RNAs from cells". Only RNA with a RIN (RNA integrity number) > 7.5 was taken forward for gene expression analysis.

DNA methylation

*Mspl*1 Reduced representation bisulphite sequencing (RRBS) was performed by Nx-Dx (now Diagenode) using their premium RRBS kit with 100ng DNA. Paired-end sequencing (2 x 50bp) was performed on an Illumina HiSeq.

Gene Expression RT-qPCR

Protocol

500ng total RNA was reverse transcribed (SuperScript® IV VILO™ cDNA Synthesis Kit, Thermo Fisher) as per the manufacturer’s instructions. RT-qPCR was performed in duplicate using 2µl 1/5 dilution cDNA. Where duplicates differed in Ct (cycle threshold) by > 0.5 the sample was repeated in triplicate. β2 microglobulin (B2M), Beta-actin, and GAPDH were used as endogenous controls. The combined mean expression (Ct) for these controls was used to normalise target mRNA expression using the ΔCt method.

Primer/probe design

Primers and probes were designed using the BioSearch RealTimeDesign Software and the guinea pig reference genome [Genome Assembly CavPor3.0: GCF_000151735.1; BioProject PRJNA12583. NCBI). Candidate gene primer/probe combinations were ordered from dnature, sequences in Table 1. Primers were used at 600nM and probes at 200nM. TaqMan assays were used for endogenous controls (B2M: Cp04183281_m1, ACTB: Cp03755212_g1, GAPDH: Cp03755742_g1), with 1µl used per reaction.

Table 1
Primers and Probes used for candidate gene expression analyses

Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'	Probe 5'-3'
CRY1	CTGTCTGTCCTGTCGGCTG	GCGCCTCGGGATTCCTATC	CGCTTTGATAAGATGGAAGGAAACCCCA
ERG	CAGGGTCACCATCAAGATGGAAT	GCCACGCTGCATTCATCAG	CCACCCTAGCCAAGTGAACGGCT
GPX4	TCTCCGCCAAGGACATTGA	CGAGGCCACATTGGTGACAA	CGGGCACATGGTCTGCCTGGATAAAT
KDM2B	CCAGCCGACAGCATCCTAC	CCGGTCCTCAGCGTTCTTC	CAAGTCGGACGACGTGCACCTG
PCCB	TGGCTGGCTACGCAGACATC	TCCTGGGTGACATCCTCATTG	TATCACTGGCCCTGATGTGGTGA
PPARA	*GGGAATCTTGCTAGCCTGTAGA*	*CCTGCCACTGAGCTGTGAC*	*AGTCCTGCTCCCAGCCGAGAAGT*
PIK3D	*TCTGGGCTCTGGAGAAACC*	*GCCTGCTCTTCAGCTCCTTAG*	*TGGCTGACACCTCTAACCTGTACC*
UBXN6	*GGCGGATCAAGCTGCAAA*	*GCCAACGGCCTCGAAGAATT*	*ACAAGGTGTTCCAGGAGCGCATCA*
VPS13D	*TCGTTGGCACCGTAACAAA*	*GTTTCCGAACCCTCTGTGCTT*	*CCGGCGCCCTGGATTTTGCTT*
FOX06	*AGGAGCTTGTCGCATGTCAG*	*AGCCCGTCCAGCAGGAGA*	*CGGCCCATACAATAACGCGAGGA*
GNAS	*TGCGCAAAGAAGCCATTGAAA*	*GCACACGTAGCCCATCTTC*	*AGCGAAGAAACGCAGCCAGCTC*

Bioinformatic/Statistical Analyses

DNA methylation

RRBS sequencing data was first processed using Bismark [21]. *Cavia porcellus* (domestic guinea pig) Genome Assembly CavPor3.0 (GCF_000151735.1) NCBI) was used. Sorted bismark alignments were further processed using Methylkit [22]; data was filtered using a minimum read count of 5 and median normalised. Only bases seen in all samples were included in the final data set for differential analysis. Differentially methylated CpG sites between adult male guinea pigs (9m) born at term vs preterm were identified and analysed as per Methylkit using logistic regression, with an overdispersion correction applied. Chi-squared test was used for statistical significance and q-value calculation for multiple testing correction.

For the identification of differentially methylated regions (DMRs) the percentage methylation data generated by Methylkit during the differential CpG analysis was analysed using Metilene [23]. DMRs were compared between groups (adult male guinea pigs born at term *vs* preterm) as per Metilene which applies both Mann-Whitney U (pMWU) and Kolmogorov-Smirnov (p2KS) tests to assess statistical significance and a q-value calculation for multiple testing correction.

Annotation information was imported from Ensembl (cporcellus_gene_ensembl) using BioMart [24,25] with hgnc, entrez ID and gene description information used and mapped against the genomic location information for the CpG and DMR regions.

Additional File 1 – Provides the basic bioinformatics workflow.

Gene expression

The combined mean expression (Ct) for the controls (β2 microglobulin (B2M), Beta-actin, and GAPDH) was used to normalise target mRNA expression using the ΔCt method ($\Delta Ct = Ct_{targetmRNA} - meanCt_{controls}$). One sample from the male term group was removed because it was a significant outlier. For each of the control genes a Ct difference of at least 4 was seen between this sample and the other samples in the group. A Mann-Whitney-U test was performed on normalised Ct values (ΔCt) to interrogate candidate gene expression between groups.

Gene enrichment analyses

These were performed using the ToppFun functional enrichment tool from the ToppGene Suite [26,27].

Results

DNA methylation

Differential methylation of CpG sites

In males we observed 743 CpG sites with significant methylation differences between term (control group) and preterm (case group) at CPNA 9m (FDR threshold < = 0.05; absolute methylation difference between groups > = 5%; Additional File 2). Of these, 316 sites were hypermethylated and 427 hypomethylated in term-born animals relative to preterm-born animals. The maximum level of hypermethylation (more methylation in controls compared to cases) observed was for a CpG which did not map to a gene (49.3%) with the second most hypermethylated CpG located in *KAT1*. The highest level of hypomethylation (less methylation in controls compared to cases) was 45.2% at a CpG site located within *PPARA*.

Of the 743 significantly differentially methylated CpGs, 672 mapped to annotated loci. Table 2 presents the top 10 of these ranked by statistical significance (A) and the top 10 ranked by absolute methylation difference (B).

Table 2
Top 10 differentially methylated CpG sites mapping to annotated loci ranked by Q value (A) and Δmethylation (B)

Scaffold: nucleotide	**Gene**	**P value**	**Q value**	**Mean Methylation Difference % Term Controls vs Preterm Cases**
A Ranked by Q value				
scaffold_15:32605089	*NFYA*	7.33E-14	4.93E-08	-29.0
scaffold_24:14808880	*RBM47*	4.79E-10	8.60E-05	38.5
scaffold_6:4192870	*PCDH1*	5.12E-10	8.60E-05	25.6
scaffold_55:676428	*C5orf49*	7.74E-10	1.04E-04	-24.6
scaffold_160:1964026	*PPARA*	9.72E-10	1.09E-04	-45.2
scaffold_17:8347073	*GABRB2*	1.63E-09	1.61E-04	-18.9
scaffold_19:15486776	*SNX19*	4.79E-09	3.07E-04	22.7
scaffold_157:283441	*MAP3K10*	4.34E-09	3.07E-04	-13.7
scaffold_42:12824725	*IL27RA*	1.40E-08	6.49E-04	-6.2
scaffold_53:8720825	*HOXB1*	1.56E-08	6.57E-04	25.3
B Ranked by Δmethylation				
scaffold_53:7878118	*KAT7*	1.86E-05	0.02946	46.7
scaffold_160:1964026	*PPARA*	9.72E-10	1.09E-04	-45.2
scaffold_216:651963	*PRR5*	3.35E-05	0.03733	-42.8
scaffold_6:11156655	*ABLIM3*	7.06E-08	0.00167	-42.4
scaffold_30:19939917	*GLI3*	4.62E-06	0.01450	42.3
scaffold_7:48813939	*KLHL6*	1.28E-05	0.02470	-41.7
scaffold_144:1075988	*ADAMTS16*	4.97E-06	0.01503	41.4
scaffold_14:16799275	*CTIF*	2.30E-08	8.62E-04	40.2
scaffold_24:14808880	*RBM47*	4.79E-10	8.60E-05	38.5
scaffold_6:12363857	*NDST1*	8.13E-06	0.01970	38.3
Differential methylation of regions				

To further investigate the differential methylation we observed between adult guinea pigs born term vs preterm we looked for extended regions of differential methylation (DMRs). We defined DMRs as regions including ≥ 5 differentially methylated CpGs and with no more than 1000 bases between CpG sites, a minimum absolute methylation difference of 10%, and filtered at a stringent threshold of q value ≤ 0.05. A total of 26 DMR met this threshold of which 24 mapped to annotated genomic loci (Table 3). Of these 24 DMRs the majority, 18/24 were

hypomethylated in the controls (term birth) vs cases (preterm birth). The DMR with the greatest CpG density (n = 21 sites) was located within *FOX06* and the largest absolute methylation difference (26.5%) was observed for a DMR in *UBXN6*.

Table 3
Significant Liver DMRs (qval <- 0.05) mapping to annotated loci in term (n = 6) vs preterm (n = 6) adult male guinea pigs

Scaffold	DMR start	DMR end	TSS* Region	DMR Size bp	CpG Number	Mean Methylation Difference % Term Controls vs Preterm Cases	qval	pMWU	p2DKS	Gene[+$]
scaffold_8	33289532	33289565		33	7	-20.3	0.0001	2.50E-08	5.73E-10	**PCCB**
scaffold_25	8927776	8927823	Y	47	7	-16.0	0.0003	1.90E-10	3.43E-09	*SYTL1*
scaffold_140	662385	662493		108	8	-16.9	0.0007	2.21E-10	1.16E-08	*SYN3*
scaffold_140	52291	52626		335	10	-19.7	0.0021	1.24E-06	5.36E-08	**CRY1**
scaffold_25	24038307	24038444		137	10	-15.7	0.0024	2.38E-06	8.33E-08	**PIK3CD**
scaffold_82	4935878	4936605	Y	727	18	-11.1	0.0031	4.86E-07	1.32E-07	*TMEM200C*
scaffold_3	60336464	60336498	Y	34	6	10.3	0.0034	8.10E-07	1.60E-07	*LOC100716779; LOC100733361*
scaffold_320	20368	20463	Y	95	7	-18.1	0.0053	1.41E-08	2.58E-07	**GPX4**
scaffold_72	7767937	7768014	Y	77	11	-16.8	0.0085	5.63E-05	4.50E-07	*FCHO1*[$]
scaffold_90	4420030	4420423		393	8	22.9	0.0100	1.19E-08	7.03E-07	**ERG**
scaffold_147	1043172	1043297		125	10	10.7	0.0106	3.31E-09	8.42E-07	*ISLR2*
scaffold_20	31204484	31204527		43	7	24.7	0.0106	7.98E-08	8.00E-07	*PTPN21*
scaffold_42	1135364	1135451		87	5	-23.6	0.0121	8.12E-09	1.05E-06	*CENPP*
scaffold_28	27090749	27090987		238	16	15.1	0.0155	5.85E-08	1.38E-06	*SCUBE1*
scaffold_509	100466	100497		31	5	-26.5	0.0208	7.89E-07	1.94E-06	**UBXN6**
scaffold_29	6251759	6251837		78	8	-11.5	0.0246	1.71E-06	2.46E-06	*LOC100731744*
scaffold_180	779209	779966		757	21	-10.2	0.0249	9.85E-09	2.56E-06	**FOXO6**
scaffold_160	1952655	1954019		1364	18	-15.3	0.0282	3.92E-12	2.96E-06	**PPARA**
scaffold_25	22053282	22053339		57	5	24.8	0.0328	8.02E-08	3.67E-06	**VPS13D**
scaffold_115	1636631	1636685		54	5	-14.4	0.0329	2.71E-08	3.76E-06	**KDM2B**
scaffold_171	1147459	1148056		597	7	-22.5	0.034204	4.38E-08	3.98E-06	*APPL2*
scaffold_2	69850166	69850279		113	11	-12.5	0.047903	2.46E-07	5.68E-06	*NFIA*

* TSS transcription start site. Promoter regions: -5000bp upstream to + 2000bp downstream of TSS. [+] bolded gene names indicate candidate genes for gene expression analyses.

Scaffold	DMR start	DMR end	TSS* Region	DMR Size bp	CpG Number	Mean Methylation Difference % Term Controls vs Preterm Cases	qval	pMWU	p2DKS	Gene[+$]
scaffold_53	7001228	7001374		146	5	-18.3	0.050853	1.93E-06	6.36E-06	*TOB1*
scaffold_25	11521349	11521506		157	5	-25.1	0.050853	1.44E-06	6.39E-06	*IFNLR1*

* TSS transcription start site. Promoter regions: -5000bp upstream to + 2000bp downstream of TSS. [+] bolded gene names indicate candidate genes for gene expression analyses.

Five DMRs were positioned within promoter regions (defined as within 5000bp upstream and 2000bp downstream of the transcription start site) suggesting potential regulatory impact.

Table 3: **Significant Liver DMRs (qval <- 0.05) mapping to annotated loci in term (n = 6) vs preterm (n = 6) adult male guinea pigs**

Pathways enrichment analyses

To explore potential functional implications, we performed pathways enrichment analyses on the genes which mapped to DMRs passing a more relaxed threshold. We applied this relaxed inclusion threshold with a nominal P value = 0.05 (Mann-Whitney U) and absolute % methylation difference of 10%, which yielded 968 DMRs mapping to annotated loci (Additional File 3), located within 788 unique genes of which 772 were recognised by ToppFun.

We focused on the later life sequelae of preterm birth looking for enrichment of genes/pathways involved in metabolic and inflammatory phenotypes which passed Bonferroni correction (adjusted P ≤ 0.05). The analyses identified 20 relevant pathways (Fig. 1) where genetic variants within the genes were associated with the disease / phenotype.

Targeted Gene Expression Analysis

Ten candidate genes were selected for expression analyses based on presence of a significant DMR (FDR < 0.05, ≥ 10% absolute DNA methylation difference), and evidence in the literature for a potential role in metabolism. The genes selected based on these criteria were *CRY1, ERG, GPX4, KDM2B, PCCB, PIK3CD, PPARA, UBXN6*, *VPS13D*, and *FOX06*.

RT-qPCR analysis for these genes was performed on RNA extracted from the same samples as the DNA used for the methylation analysis, one sample from the term group was removed from the analysis as an outlier (see methods for details). Gene expression was detected for 9/10 genes interrogated, and a significant difference in mRNA expression for *CRY1* (P = 0.004) observed, with a greater level of *CRY1* expression seen in the term control group compared to the preterm group (Table 4). This finding aligns with the DMR analysis where hypermethylation of *CRY*1 was observed in the preterm compared to term group (mean methylation difference 19.7%, adjusted P 0.0021, Table 3); this is consistent with the canonical inverse relationship between methylation and gene expression.

No mRNA expression was detected for *FOXO6* in any of the samples. It should be noted that primer design relied on the CavPor3.0 reference assembly (GCF_000151735.1), which at the time was relatively fragmented (3,143 scaffolds), potentially limiting the accuracy of exon boundary definition and primer placement. Subsequent work where the primers and probe sequences for *FOXO6* (Table 1) were compared against the current *Cavia porcellus* genome assembly (mCavPor4.1; GCF_034190915.1) revealed mismatches and non-alignment with *FOX06*.

Next, we expanded our gene expression analyses to the livers of adult (9m) female guinea pigs born preterm or at term, examining the 9 genes for which we observed expression in the male guinea pigs. We did not observe any statistically significant differences in expression for any of the 9 candidate genes in the female guinea pigs (Table 4).

Given our observation of differential expression of *CRY*1 between the preterm and term groups in males but not in females, we investigated whether the level of expression for each candidate differed between the equivalent male and female groups. Table 4 presents a summary of this. For the term guinea pigs a comparison between males and females revealed statistically significant differences in the mRNA expression of *CRY*1 and *VPS13D*, we also observed differential expression between males and females for *VPS13D* in the preterm group animals, as well as for *PIK3CD*.

Table 4

Mean normalised expression (ΔCt) for nine candidate genes in the livers of adult male and female guinea pigs born at term and preterm.

Gene	Male			Female			Term M:F	Preterm M:F
	Preterm N = 6	Term N = 5	P-value	Preterm N = 5	Term N = 6	P-value	P-value	P-value
CRY1	6.64 ± 0.25	6.10 ± 0.15	**0.004**	6.44 ± 0.40	6.59 ± 0.12	0.54	**0.008**	0.42
ERG	7.97 ± 0.14	8.12 ± 0.14	0.12	7.71 ± 0.44	7.80 ± 0.27	0.79	0.095	0.30
GPX4	2.38 ± 0.27	2.23 ± 0.16	0.43	2.13 ± 0.32	2.24 ± 0.11	0.66	1	0.20
KDM2B	9.53 ± 0.29	9.49 ± 0.53	0.93	9.66 ± 0.32	9.55 ± 0.25	0.93	1	0.75
PCCB	4.40 ± 0.13	4.38 ± 0.16	1	4.39 ± 0.22	4.34 ± 0.05	0.43	0.55	1
PIK3CD	11.41 ± 0.36	11.29 ± 0.65	1	10.85 ± 0.30	10.69 ± 0.34	0.66	0.22	**0.026**
PPARA	7.48 ± 0.35	7.74 ± 0.26	0.14	7.55 ± 0.24	7.52 ± 0.16	0.54	0.31	0.39
UBXN6	7.59 ± 0.61	7.35 ± 0.26	0.33	6.99 ± 0.32	6.96 ± 0.49	0.93	0.31	0.13
VPS13D	8.97 ± 0.25	8.93 ± 0.22	1	8.28 ± 0.43	8.36 ± 0.30	0.66	**0.016**	**0.015**

Discussion

This proof of principle study provides, to our knowledge, the first genome-wide analysis of DNA methylation in the liver following preterm birth in any species. Previous epigenetic studies following preterm birth in humans have focused on accessible tissues such as cord blood, placenta, peripheral blood or buccal cells [3–9]. While informative, such tissues cannot directly interrogate metabolically active organs that mediate long-term disease risk. By interrogating the adult hepatic methylome in a translational guinea pig model, we demonstrate that preterm birth leaves persistent, organ-specific epigenetic marks. Crucially, too, this approach has allowed separation of the impact of gestational age from other factors influencing both fetal and postnatal life in those born preterm.

The liver plays a critical role in metabolism. As such we hypothesised that given the increased risk of metabolic complications in later life associated with preterm birth there would be significant epigenetic differences in the livers of guinea pigs between term- and preterm-born individuals. Our observations support this hypothesis. We identified 743 differentially methylated CpGs (Table 2; Additional File 2) and 24 DMRs (Table 3) between adult male guinea pigs born preterm and their term-born counterparts, indicating substantial and sustained epigenetic remodelling.

Among ten candidate genes selected for transcriptional validation, *CRY1* emerged as a convergent finding, exhibiting increased methylation and reduced mRNA expression in preterm-born males. This gene encodes a transcriptional repressor involved in the regulation of circadian rhythm. Circadian control of hepatic metabolism is well-established; *CRY1* modulates hepatic gluconeogenesis and integrates insulin/SREBP1c signalling and protein turnover (DDB1/CUL4A; autophagic degradation), situating *CRY1* at the interface of clock and glucose homeostasis [28–31]. Disruption of circadian rhythm biology in mammals is associated with several conditions including obesity, insulin resistance and metabolic syndrome. Additionally, we observed sex-specific differential expression of *CRY1* whereby mRNA expression was significantly altered only in males (Table 4); this aligns with emerging evidence of sexual dimorphism in the circadian hepatic transcriptome, where males and females differ markedly in rhythmic gene expression and metabolic pathway activation [32], and with broader sex-dependent differences in hepatic metabolism, shaped by hormone-driven regulation and circadian signalling [33]. These observations also mirror epidemiologic evidence that sex influences complications associated with being born preterm, including long-term outcomes [34–37]. Collectively, this highlights the importance of sex-stratified analyses in future epigenetic programming research and together, these findings position *CRY1* as a mechanistic bridge linking circadian regulation, hepatic glucose metabolism, and the long-term metabolic risk observed following preterm birth.

It is not uncommon for significant differences in DNA methylation not to correlate with observed changes in gene expression – especially as measured here using targeted gene expression assays. There are several potential reasons for this, including that DNA methylation canregulate alternate splicing [38, 39]) and if the affected isoform is not targeted by the assay no difference in mRNA expression would be observed, Furthermore, the role of DNA methylation in gene regulation is incompletely understood and complex. For instance, while the classic observation that DNA methylation within promoter regions leads to down-regulation of gene expression [40] holds in certain cases, most promoters are not regulated by DNA methylation [41]. While variations in DNA methylation within gene bodies can also regulate gene expression [42], and loss of methylation can activate enhancers located in intronic regions [43]. Also, DNA methylation does not act in

isolation; cross-talk and coordination between it and other epigenetic mechanisms, such as histone lysine methylation [44], work together in gene regulation and genomic function. In addition, changes in DNA methylation detected at the tissue level may be representative of cell specific changes which are not detectable at the tissue global RNA level.

Therefore, looking beyond *CRY1*, where we observed the change in DNA methylation, significant DMRs converged on interconnected metabolic pathways central to hepatic resilience. Furthermore, the pathways enrichment analysis of 788 unique genes mapping to 968 DMRs passing a more relaxed threshold (nominal P value = 0.05, absolute % methylation difference 10%; Additional File 3) identified enrichment for genes involved in a number of sequalae associated with the long-term disease risk of preterm birth, such as cardiovascular and coronary artery disease, blood lipid and glucose, blood pressure, and liver cirrhosis (Fig. 1).

PPARA, where we observed the largest CpG-level of hypermethylation (45.2%, Table 2) in cases (preterm) compared to controls (term), and a significant DMR (1364bp, 18 CpGs, 15.3% methylation difference) encodes PPARα, a master transcriptional regulator; this transcription factor is involved in the regulation of fatty acid oxidation and ketogenesis, and its activity protects against fasting-induced lipid accumulation, hepatic steatosis, and metabolic dysfunction [45–49]. Similarly, *GPX4*, for which we also observed significant hypermethylation in the preterm group (DMR: 95bp, 7 CpGs, 18.1% methylation difference, Table 3), encodes phospholipid hydroperoxide glutathione peroxidase, which plays a pivotal role in suppressing ferroptosis and maintaining lipid homeostasis; with perturbations in GPX4 signalling linked to oxidative lipid injury and cardiometabolic disease [50–52].

Autophagy plays a critical role in maintaining hepatic metabolic homeostasis via regulation of organelle turnover, lipid droplet degradation, and protein quality control [53]. *UBXN6* (DMR: 31bp, 5CpGs, 26.5% hypermethylation in preterm group, Table 3) encodes UBXD1, which is essential for autophagy induction [54]. *VPS13D* (DMR: 57bp, 5 CpGs, 24.8% hypomethylation in preterm group, Table 3), is a lipid-transfer protein at organelle contact sites and required for completion of mitophagy [55]. Impaired autophagy/mitophagy represents a potential source of oxidative stress and metabolic vulnerability, implicating it in the pathogenesis of MASLD/MASH (metabolic dysfunction-associated steatotic liver disease/metabolic dysfunction associated steatohepatitis), insulin resistance, and other metabolic disorders, due to the defective clearance of damaged mitochondria and accumulated lipids which promote oxidative stress and inflammation [56, 57]. Therefore, differential methylation at *UBXN6* and *VPS13D* may signal a disruption in autophagic and mitophagic capacity, potentially reducing the liver's ability to maintain energy balance and respond to metabolic challenges - a plausible mechanism linking preterm birth to long-term hepatic vulnerability which warrants further investigation.

The potential interplay of other epigenetic mechanisms in hepatic disruption is suggested by *KDM2B* (a JmjC histone demethylase targeting H3K36me2/H3K4me3). KDM2B works in concert with the EZH2/PRC2 pathway, which deposits H3K27me3 to silence metabolic and fibrogenic gene networks. Emerging evidence places EZH2 at the center of NAFLD/NASH progression: EZH2 activity increases with chronic liver injury, promotes stellate-cell activation and fibrosis, and its pharmacologic inhibition reduces inflammatory cytokines and fibrosis markers in experimental NASH while reprogramming H3K27me3 across pro-fibrotic pathways [58, 59]. Given KDM2B's role in lineage specification [60,61] and chromatin remodeling [62], preterm-associated hypermethylation (DMR 54bp, CpGs 5, % methylation difference 14.4) at *KDM2B* could potentially influence hepatocyte and non-parenchymal cell transcriptional states toward lipid handling, redox balance, and extracellular-matrix signaling, thereby increasing susceptibility to NAFLD-like injury [63,64].

PIK3CD encodes PI3K-δ; a leukocyte-enriched PI3K catalytic subunit which is the dominant PI3K isoform in hepatic sinusoids, principally Kupffer cells and monocyte-derived macrophages. During liver injury and MASLD/MASH, changes in Kupffer-cell identity, monocyte recruitment, and inflammatory tone shape paracrine crosstalk with endothelium and hepatocytes, thereby modulating insulin/lipid pathways and fibrogenic progression [65,66]. Although hepatocyte insulin signalling is largely mediated by PI3K-α/β upstream of AKT, the immune compartment's PI3K-δ activity contributes to the local cytokine milieu and innate immune responses that influence lipogenesis, fatty-acid oxidation, and glucose output [67].

Clinical and experimental metabolomics consistently report amino-acid signatures in MASLD/MASH such as increased valine/isoleucine/glutamate/tyrosine, and decreased glycine/serine/arginine), linked to energy metabolism (including glycolysis, gluconeogenesis, TCA cycle, and mitochondrial transfer), amino acid metabolism, fatty acid oxidation, and methylation and detoxification pathways [68–70]. *PCCB* where we observed the most statistically significant DMR (Table 3) encodes the β-subunit of propionyl-CoA carboxylase, a biotin-dependent mitochondrial enzyme that channels carbon from branched-chain amino acids and odd-chain fatty acids into the TCA cycle by converting propionyl-CoA to methylmalonyl-CoA and, subsequently, to succinyl-CoA, thereby replenishing TCA-cycle intermediates and supporting cellular redox homeostasis.

Collectively, these findings support a coordinated model in which preterm birth reshapes hepatic epigenetic architecture across interconnected regulatory layers: circadian timing (*CRY1*), lipid oxidation and redox buffering (*PPARA*, *GPX4*), mitochondrial quality control (*UBXN6*, *VPS13D*), chromatin remodeling (*KDM2B*/*EZH2*), immune tone (*PIK3CD*), and carbon flux through the TCA cycle (*PCCB*). Rather than isolated gene effects, this pattern suggests systems-level recalibration of hepatic metabolic resilience.

A key strength of this study is the organ-specific analysis in adult liver within a precocial species whose *in utero* development and placentation share similarities with humans. Limitations include modest sample size and reliance on an earlier draft guinea pig reference assembly, which constrained annotation and primer design. However, the identification of robust differential methylation and convergent methylation–expression changes at *CRY1* supports the biological validity of the findings. Recent improvements in guinea pig genome assemblies will further enhance mechanistic interrogation in future work.

Conclusions

This study provides the first epigenetic analysis of adult liver following preterm birth and demonstrates persistent genome-wide methylation differences enriched at metabolic regulatory loci. The pattern of differential methylation converges on circadian, mitochondrial/autophagic, vascular, immune, and chromatin pathways, with *CRY1* offering a focal mechanistic link between circadian regulation and hepatic metabolism. Together, these data support a model in which early premature transition to the extrauterine environment induces durable epigenetic reprogramming of hepatic metabolic networks, potentially increasing susceptibility to later-life metabolic disease. Future multi-omic, cell-type–resolved, and sex-stratified studies incorporating functional validation will be essential to test causality and identify potential targets.

Declarations

Ethics approval and consent to participate

All animal use was prospectively approved by the University of Otago Wellington Animal Ethics Committee (AUP 8-14; AUP 18-214) and conformed to Health Research Council of New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. Reporting follows ARRIVE 2.0 guidelines [71]. The experimental unit was the individual animal.

Consent for publications

Not applicable

Availability of data and materials

RRBS data is available at NCBI SRA accession number PRJNA1470843. Please note embargoed until publication.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

DM, MCB, RMD and MB conceived and designed the study, JC carried out sample preparation and gene expression analyses, animal husbandry was performed by RMD, and HB, bioinformatic analyses was performed by MCB and DM, and statistical analyses were carried out by GF. All authors read and approved the manuscript.

References

1. 1. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nat Rev Genet. 2013;14:204–20.
2. 2. Chen M, Zhang L. Epigenetic mechanisms in developmental programming of adult disease. Drug Discov Today. 2011;16:100–7.

3. 3. Hodge KM, Burt AA, Camerota M, Carter BS, Check J, Conneely J et al. Epigenetic associations with neonatal age in infants born very preterm, particularly among genes involved in neurodevelopment. Sci Rep. 2024;14:
4. 4. Merid SK, Novoloaca A, Sharp GC, Küpers LK, Kho AT, Roy R et al. Epigenome-wide meta-analysis of blood DNA methylation in newborns and children identifies numerous loci related to gestational age. Genome Med. 2020;12:1–15.
5. 5. Spada E, Calzari L, Corsaro L, Fazia T, Mencarelli M, Di Blasio AM et al. Epigenome Wide Association and Stochastic Epigenetic Mutation Analysis on Cord Blood of Preterm Birth. Int J Mol Sci. 2020;21:5563.
6. 6. Ahmad F, Lakshmanan AP, Alabduljabbar S, Ahmed SH, Ahmed A, Kabeer BSA et al. Placental and cord blood DNA methylation in preterm birth: exploring the epigenetic role of maternal dietary protein. NPJ Sci Food. 2025;9:56.
7. 7. Andrews SV, Yang IJ, Froehlich K, Oskotsky T, Sirota M. Large-scale placenta DNA methylation integrated analysis reveals fetal sex-specific differentially methylated CpG sites and regions. Sci Rep. 2022;12:3456.
8. 8. Everson TM., O'Shea, TM, Burt, A. Hermetz K, Carter BS, Helderman *et al.* Serious neonatal morbidities are associated with differences in DNA methylation among very preterm infants. Clin Epigenet. 2020; 12:151.
9. 9. Camerota M, Lester BM, Castellanos FX, Carter BS, Check J, Helderman J *et al.* Epigenome-wide association study identifies neonatal DNA methylation associated with two-year attention problems in children born very preterm. Transl Psychiatry.2024;14:126.
10. 10. Kuula J, Czamara D, Hauta-alus H, Lahti J, Hovi P, Miettinen ME et al. Epigenetic signature of very low birth weight in young adult life. Pediatr Res. 2024;97:123–30.
11. 11. Islam MDR, Hanif A, Wong YT, Silk, TJ, Craig JM. Association between DNA methylation at birth and cognitive outcomes from early childhood to adolescence: A systematic review. Neurosci Biobehav Rev. 2026;181:106405.
12. 12. Bianco-Miotto T, Craig JM, Gasser YP, van Dijk SF, Ozanne SE. Epigenetics and DOHaD: from basics to birth and beyond. J Dev Orig Health Dis. 2017;8:266–75.
13. 13. Morrison JL, Botting KJ, David AL, Dyson RM, Garford KL, Gray C et al. Guinea pig models for translation of the developmental origins of health and disease hypothesis into the clinic. J Physiol. 2018;596:5535–69.
14. 14. Berry M, Gray C, Wright K, Dyson R, Wright I. Premature guinea pigs: a new paradigm to investigate the late-effects of preterm birth. J Dev Orig Health Dis. 2015;6:143–8.
15. 15. Shaw JC, Palliser HK, Dyson RM, Berry MJ, Hirst J. Disruptions to the cerebellar GABAergic system in juvenile guinea pigs following preterm birth. Int J Dev Neurosci. 2017;65:1–8.
16. 16. Shaw JC, Dyson RM, Palliser HK, Gray C, Berry MJ, Hirst JJ. Neurosteroid replacement therapy using the allopregnanolone-analogue ganaxolone following preterm birth in male guinea pigs. Pediatr Res. 2019;85:86–96.
17. 17. Shaw JC, Dyson RM, Palliser HK, Sixtus RP, Barnes H, Pavy CL et al. Examining neurosteroid-analogue therapy in the preterm neonate for promoting hippocampal neurodevelopment. Front Physiol. 2022;13:820123.
18. 18. Moloney RA, Palliser HK, Dyson RM, Carlton L, Pavy CL, Berry MJ et al. Ongoing effects of preterm birth on the dopaminergic and noradrenergic pathways in the frontal cortex and hippocampus of guinea pigs. Dev Neurobiol. 2024;84:45–60.
19. 19. Pavy CL, Shaw JC, Dyson RM, Palliser HK, Moloney RA, Sixtus RP et al. Ganaxolone therapy after preterm birth restores cerebellar oligodendrocyte maturation and myelination in guinea pigs. Dev Psychobiol. 2024;66:e22345.
20. 20. Sixtus RP, Gray C, Barnes H, Paterson ESJ, Berry MJ, Dyson RM. Cardiovascular responses to heat and cold exposure are altered by preterm birth in guinea pigs. Physiol Rep. 2024;12:e15876.
21. 21. Krueger F, Andrews SR. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics. 2011;27:1571–2.
22. 22. Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A et al. methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. Genome Biol. 2012;13:R87.
23. 23. Jühling F, Kretzmer H, Bernhart SH, Otto C, Stadler PF, Hoffman S. metilene: fast and sensitive calling of differentially methylated regions from bisulfite sequencing data. Genome Res. 2016;26:256–62.
24. 24. Durinck S, Spellman PT, Birney E, Huber W. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nat Protoc. 2009;4:1184–91.
25. 25. Durinck S, Moreau Y, Kasprzyk A, Davis S, Moor B, Brazma A, Huber W. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. Bioinformatics. 2005;21:3439–40.
26. 26. Chen J, Bardes EE, Aronow BJ, Jegga AG. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. 2009;37:W305–11.
27. 27. Chen J, Aronow BJ, Jegga AG. Disease candidate gene identification and prioritization using protein interaction networks. BMC Bioinformatics. 2009;10:73.

28. 28. Tong X, Zhang D, Charney N, Jin E, Van Dommelen K, Stamper K et al. DDB1-mediated CRY1 degradation promotes FOXO1-driven gluconeogenesis in liver. Diabetes. 2017;66:1013–21.
29. 29. Jang H, Lee G, Kim JBl. SREBP1c-CRY1 axis suppresses hepatic gluconeogenesis upon insulin. Cell Cycle. 2016;16:123–30.
30. 30. Toledo M, Batista-Gonzalez A, Merheb E, Aoun ML, Tarabra E, Daorong F et al. Autophagy regulates the liver clock and glucose metabolism by degrading CRY1. Cell Metab. 2018;28:268–81.
31. 31. Rajan PK, Udoh U-AS, Finley R, Pierre SV, Sanabria J. The biological clock of liver metabolism in metabolic dysfunction-associated steatohepatitis progression to hepatocellular carcinoma. Biomedicines. 2024;12:456.
32. 32. Astafev AA, Mezhnina V, Poe A, Jiang P, Kondratov RV. Sexual dimorphism of circadian liver transcriptome. iScience. 2024;27:109999.
33. 33. Kočar E, Cokan KB, Kreft T, Režen T, Rozman D. The importance of sex dimorphism in liver metabolism and progressive liver diseases. Biol Sex Differ. 2025;16:12.
34. 34. Yoshida-Montezum Y, Sivapathasundaram B, Brown HK, Keown-Stoneman C, de Souza RJ, To T et al. JAMA Netw Open. 2022;5:e2214379.
35. 35. Crump C, Winkleby MA, Sundquist K, Sundquist J. Risk of hypertension among young adults who were born preterm: a Swedish national study of 636,000 births. Am J Epidemiol. 2011;173:797–803.
36. 36. Crump C, Sundquist J, Sundquist K. Association of preterm birth with lipid disorders in early adulthood: a Swedish cohort study. PLoS Med. 2019;16:e1002947.
37. 37. Gette F, Ali SA, Ho MSP, Richter LL, Chan ES, Yang CL Long-term health outcomes of preterm birth: a narrative review. Front. Pediatr. 2025;13:1565897.
38. 38. Song K, Li L, Zhang G. The association between DNA methylation and exon expression in the Pacific oyster *Crassostrea gigas*. PloS One. 2017; 12:e0185224
39. 39. Shayevitch R, Askayo D, Keydar I. The importance of DNA methylation of exons on alternative splicing AST G. RNA. 2018;24:1351–1362
40. 40. Boyes J, Bird A. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. Cell. 1991;64:1123–1134
41. 41. Mattei AL, Bailly N, Meissner A. DNA methylation: a historical perspective. Trends in Genetics. 2022;38:676–707.
42. 42. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012;13:484–492.
43. 43. Blattler A, Yao L, Witt H, Guo Y, Nicolet CM, Berman BP et al. Global loss of DNA methylation uncovers intronic enhancers in genes showing expression changes. Genome Biology. 2014;15:469
44. 44. Tibben BM, Rothbart SB. Mechanisms of DNA methylation regulatory function and crosstalk with histone lysine methylation. J Mol. Biol. 2024;1:436(7).
45. 45. Kersten S. Integrated physiology and systems biology of PPARα. Mol Metab. 2014;3:354–71.
46. 46. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. J Hepatol. 2015;62:720–33.
47. 47. Lin Y, Wang Y, Li P-F. PPARα: an emerging target of metabolic syndrome, neurodegenerative and cardiovascular diseases. Front Endocrinol. 2022;13:845321.
48. 48. Régnier M, Polizzi A, Smati S, Lukowicz C, Fougerat A, Lippi Y et al. Hepatocyte-specific deletion of Pparα promotes NAFLD in the context of obesity. Sci Rep. 2020;10:113.
49. 49. Todisco S, Santarsiero A, Convertini P, De Stefano G, Gilio M, Iacobazzi V, Infantino V. PPAR alpha as a metabolic modulator of the liver: role in the pathogenesis of nonalcoholic steatohepatitis (NASH). Biology (Basel). 2022;11:567.
50. 50. Zhang JB, Tong J, Sun DY, Fu JT, Li DJ, Wang P. Targeting ferroptosis in cardio-metabolic diseases: mechanisms and therapeutic prospects. Med Res Rev. 2023;43:210–45.
51. 51. Kim JW, Lee JY, Oh M, Lee EW. An integrated view of lipid metabolism in ferroptosis revisited via lipidomic analysis. Exp Mol Med. 2023;55:567–80.
52. 52. Zheng J, Conrad M. Ferroptosis: when metabolism meets cell death. Physiol Rev. 2025;105:789–900.
53. 53. Ueno T, Komatsu M. Autophagy in the liver: functions in health and disease. Nat Rev Gastroenterol Hepatol. 2017;14:170–84
54. 54. Madsen L, Andersen KM, Prag S, Moos T, Semple CA, Seeger M. Ubxd1 is a novel co-factor of the human p97 ATPase. Int J Biochem Cell Biol. 2008;40:2927–42.
55. 55. Insolera R, Lőrincz P ,Wishnie AJ, Juhász G, Collins CA. Mitochondrial fission, integrity and completion of mitophagy require separable functions of Vps13D in Drosophila neurons. PLoS Genet. 2021;17:e1009368.
56. 56. Liu Y, Zhang M, Wang Y. Induction of autophagy as a therapeutic breakthrough for NAFLD: current evidence and perspectives. Biology (Basel). 2025;14:123.

57. 57. Sadeghi A, Niknam M, Momeni-Moghaddam MA, Shabani M, Aria H, Bastin A et al. Crosstalk between autophagy and insulin resistance: evidence from different tissues. Eur J Med Res. 2023;28:234.
58. 58. Lim HJ, Kim M. EZH2 as a potential target for NAFLD therapy. Int J Mol Sci. 2020;21:1234.
59. 59. Li XJY, Zhou F, Li YJ, Xue XY, Qu JR, Fan GF et al. LncRNA H19-EZH2 interaction promotes liver fibrosis via reprogramming H3K27me3 profiles. Acta Pharmacol Sin. 2023;44:789–800.
60. 60. He J, Shen L, Wan M, Taranova O, Wu H, Zhang Y. Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes. Nat Cell Biol. 2013;15:1165–75.
61. 61. Andricovich J, Kai Y, Peng W, Foudi A, Tzatsos A. Histone demethylase KDM2B regulates lineage commitment in normal and malignant hematopoiesis. J Clin Invest. 2016;126:203–13.
62. 62. Jones BA, Varambally S, Arend RC. Histone methyltransferase EZH2: a therapeutic target for ovarian cancer. Mol Cancer Ther. 2018;17:987–96.
63. 63. Shi Y, Qi W. Histone modifications in NAFLD: mechanisms and potential therapy. Int J Mol Sci. 2023;24:5678.
64. 64. Vacík T, Lađinović D, Raška I. KDM2A/B lysine demethylases and their alternative isoforms in development and disease. Nucleus. 2018;9:357–68.
65. 65. Li W, Chang N, Li L. Heterogeneity and function of Kupffer cells in liver injury. Front Immunol. 2022;13:789456.
66. 66. Uehara K, Santoleri D, Whitlock AEG, Titchenell PM. Insulin regulation of hepatic lipid homeostasis. Compr Physiol. 2023;13:345–60.
67. 67. Molinaro A, Becattini B, Mazzoli A, Bleve A, Radici L, Maxvall I et al. Insulin-driven PI3K-AKT signaling in the hepatocyte is mediated by redundant PI3Kα and PI3Kβ activities and is promoted by RAS. Cell Metab. 2019;29:123–35.
68. 68. Masoodi M, Gastaldelli A, Hyötyläinen T, Arretxe E, Alonso C, Gaggini M et al. Metabolomics and lipidomics in NAFLD: biomarkers and non-invasive diagnostic tests. Nat Rev Gastroenterol Hepatol. 2021;18:675–90.
69. 69. Piras C, Noto A, Ibba L, Deidda M, Fanos V, Muntoni S et al. Contribution of metabolomics to the understanding of NAFLD and NASH syndromes: a systematic review. Metabolites. 2021;11:345.
70. 70. Mudgal P, Gupta SK, Malik S, Nith RB, Kumar S, Chaturvedi RKK et al. Biomarker discovery in NAFLD: insights from metabolomics and vote counting meta-analysis. Metabolomics. 2025;21:89.
71. 71. Kilkenny C, Browne WJ, Cuthill IA, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Osteoarthritis Cartilage. 2012;20:256–60.

Figures

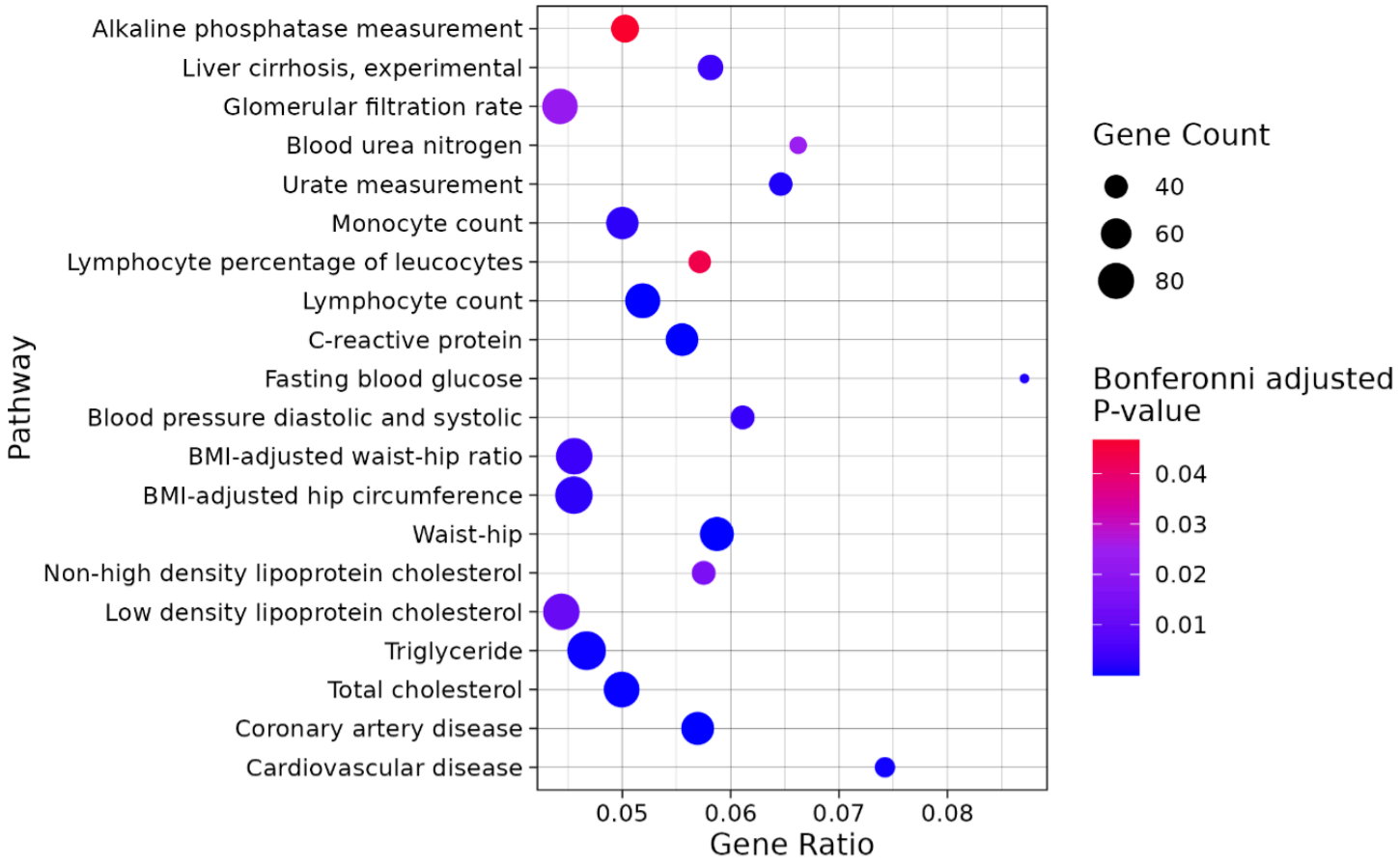


Figure 1

Enrichment of genes involved in metabolic and inflammatory phenotpyes for DMR loci (772 genes) passing adjusted P ≤ 0.05.

Supplementary Files

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- AdditionalFile2CpGsfdr05Abs5.xlsx
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