Wu et al. BMC Molecular and Cell Biology (2024) 25:19
https://doi.org/10.1186/s12860-024-00515-7

BMC Molecular and Cell Biology

RESEARCH

Open Access

Differential methylation patterns in paternally imprinted gene promoter regions in sperm from hepatitis B virus infected individuals

Baoyan Wu[1,2,3†], Yuying Sheng[1,2,3†], Wenwei Yu[1,2,3†], Lewen Ruan[1,2,3], Hao Geng[1,2,3], Chuan Xu[1,2,3], Chao Wang[1,2,3], Dongdong Tang[1,2,3], Mingrong Lv[1,2,3*], Rong Hua[2,3*] and Kuokuo Li[1,2,3*]

Abstract

Background Hepatitis B virus (HBV) infection poses a substantial threat to human health, impacting not only infected individuals but also potentially exerting adverse effects on the health of their offspring. The underlying mechanisms driving this phenomenon remain elusive. This study aims to shed light on this issue by examining alterations in paternally imprinted genes within sperm.

Methods A cohort of 35 individuals with normal semen analysis, comprising 17 hepatitis B surface antigen (HBsAg)-positive and 18 negative individuals, was recruited. Based on the previous research and the Online Mendelian Inheritance in Man database (OMIM, https://www.omim.org/), targeted promoter methylation sequencing was employed to investigate 28 paternally imprinted genes associated with various diseases.

Results Bioinformatic analyses revealed 42 differentially methylated sites across 29 CpG islands within 19 genes and four differentially methylated CpG islands within four genes. At the gene level, an increase in methylation of *DNMT1* and a decrease in methylation of *CUL7*, *PRKAG2*, and *TP53* were observed. DNA methylation haplotype analysis identified 51 differentially methylated haplotypes within 36 CpG islands across 22 genes.

Conclusions This is the first study to explore the effects of HBV infection on sperm DNA methylation and the potential underlying mechanisms of intergenerational influence of paternal HBV infection.

Keywords Hepatitis B virus, Imprinted gene, Paternally, Methylation, Sperm

†Baoyan Wu, Yuying Sheng and Wenwei Yu contributed equally to this work and share first authorship.

*Correspondence:
Mingrong Lv
lmr2010@mail.ustc.edu.cn
Rong Hua
ahmuhuarong@126.com
Kuokuo Li
likuokuo_bio@163.com
[1] Reproductive Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Anhui Medical University, No. 218 Jixi Road, Hefei 230022, China
[2] NHC Key Laboratory of Study On Abnormal Gametes and Reproductive Tract (Anhui Medical University), Key Laboratory of Population Health Across Life Cycle (Anhui Medical University), Ministry of Education of the People's Republic of China, Hefei, Anhui, China
[3] Key Laboratory of Population Health Across Life Cycle (Anhui Medical University), Ministry of Education of the People's Republic of China, Hefei, Anhui, China

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Background

Hepatitis B virus (HBV) infection represents a substantial global health concern, with an estimated 30% of the world's population having encountered the virus at some point in their lives, notwithstanding the implementation of preventive vaccines over several decades. Recognized as a significant threat to human health and a prominent cause of mortality, HBV infection remains a top health priority [1, 2].

While the primary mode of HBV transmission is vertical, occurring from mother to neonate. HBV infection in pregnant females have been clearly related to miscarriage. Infection with HBV has been linked to a higher risk of miscarriage and preterm birth in natural pregnancies [3–5]. Both horizontal and vertical father-to-child transmission are also acknowledged as pivotal contributors [6–9]. The potential for sperm-mediated intrauterine HBV infection has stirred considerable controversy. Some evidence suggests the integration of HBV-DNA into the chromatin of human sperm, and a small-scale study employing direct sequencing has indicated paternal HBV transmission to the fetus via sperm [10–12]. However, a larger body of evidence contradicts this, revealing that a father's carrier state is unlikely to result in fetal HBV infection, as evidenced by the absence of HBV DNA in 164 fetuses from the same study born to HBV-positive fathers and HBV-negative mothers [13].

Beyond the controversies surrounding vertical transmission, the adverse impact of paternal HBV infection on sperm quality, chromosomal stability, and pregnancy outcomes in assisted reproductive technology (ART) has been extensively documented [10, 14, 15]. Notably, Lorusso et al. reported significant decreases in sperm count, motility, viability, and normal morphology in HBV seropositive patients, while Huang et al. identified higher chromosomal aberrations in sperm from hepatitis B patients, suggesting potential adverse effects on chromosomal stability [10, 15]. Furthermore, a meta-analysis conducted by Xiong et al. revealed reduced clinical pregnancy and live birth rates per cycle in couples undergoing ART where the male partner was exclusively infected with HBV [14]. Moreover, the influence of paternally transmitted HBV infection extends to postnatal health, as evidenced by a cohort study in Taiwan, indicating a significantly increased incidence of hepatoblastoma in children born to fathers with HBV infection [16].

The repercussions on sperm quality and ART pregnancy outcomes attributable to HBV infection may be linked, in part, to sperm chromosomal aberrations and altered CpG methylation both before and after sperm maturation and fertilization [10, 14, 17]. Moreover, HBV infection in pregnant females, can also influence the methylation patterns of the offspring [18]. In a study by Qijun Cheng et al., it was found that prenatal HBV exposure, even in the absence of malformations or prematurity, may alter the epigenomic profile of newborns [19]. Yet, there exists a limited understanding of how paternal HBV infections lead to adverse fetal health outcomes, including the rarely attributed occurrence of hepatoblastoma. The present study seeks to elucidate potential underlying mechanisms through a novel exploration of the perspective of paternally imprinted genes, shedding light on how paternal HBV infection may modulate offspring health.

Materials and methods

Participants and study design

A total of 35 subjects from the Reproductive Center of the First Affiliated Hospital of Anhui Medical University (Hefei, China) were enrolled in this study. All participants underwent in vitro fertilization due to female causes of infertility at this center. Of the 35 patients (Supplementary Dataset 1), 17 were positive for hepatitis B surface antigen (HBsAg) and constituted the case group, while the remaining 18 individuals, negative for HBsAg, were recruited as controls (Fig. 1 illustrates the study workflow) [1].

The inclusion criteria of the study entailed: (I) patient age between 22–40 years; (II) normal results of routine semen analysis and sperm morphology according to the sixth edition of the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen [20]; (III) no history of any mental disease or somatic severe disorder; (IV) and no history of smoking, excessive alcohol use, drug consumption, etc.

Semen sample processing

All individuals were required to provide a semen sample by masturbation that was collected after a previous ejaculation that occurred 3–7 days ago. Subsequently, semen samples were frozen at -80℃. Semen samples underwent washing with 10 mL of 1X phosphate-buffered saline, followed by centrifugation and resuspension of the pellet in 10 mL of somatic cell lysis buffer (0.1% SDS, 0.5% Triton X-100 in DEPC H2O; Sigma-Aldrich). Overnight incubation at 4℃ was followed by multiple centrifugations and resuspension of the pellet in 10 mL phosphate-buffered saline.

Selection of genes

According to Tucci's article, there are more than 200 imprinted genes [21], and in order to further evaluate the correlation between paternally inherited genes and human genetic diseases, we searched for genes among these 200 genes that may be associated with the occurrence of human genetic diseases. A detailed list of these

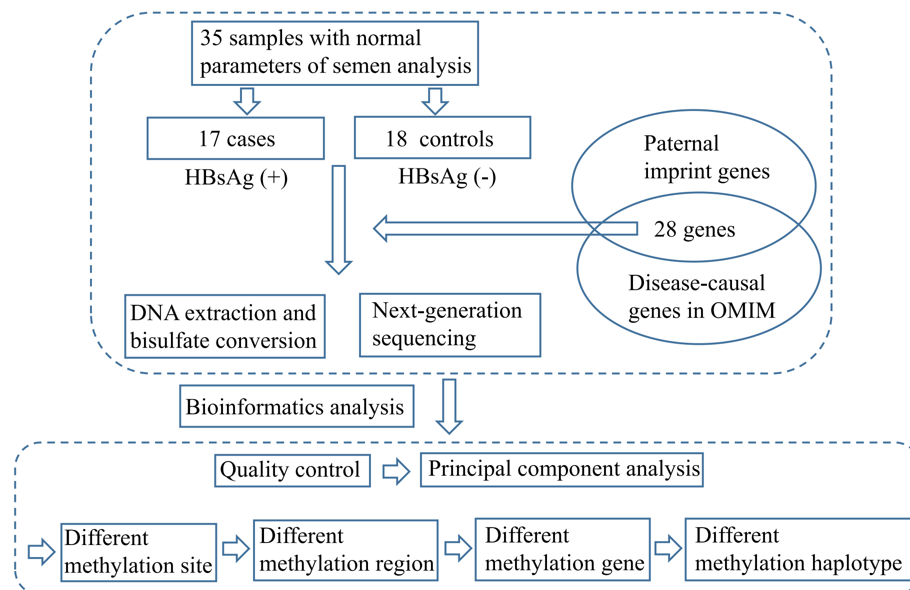

Fig. 1 Workflow of this different methylation study. Hbs Ag: hepatitis B surface antigen; Hbs Ag (+): hepatitis B surface antigen positive; Hbs Ag (-): hepatitis B surface antigen negative

genes and their related functional information is provided in Supplementary Dataset 2.

DNA methylation sequencing and bioinformatics analyses

Given the crucial role of CpG island methylation in influencing gene expression and functionality, we analyzed methylation differences at different levels from CpG sites, CpG island regions and individual genes. Therefore our investigation focused on CpG islands within potential promoter regions spanning + 2000 bp to -1000 bp of the transcriptional start site. Putative CpG islands were predicted using EMBOSS Explore, with regions having an observed/expected ratio exceeding 0.6, a length surpassing 200 bp, and a GC content exceeding 50% defined as CpG islands. These islands underwent targeted methylation sequencing.

Sperm DNA underwent treatment using the sodium bisulfite-based EZ DNA Methylation-Gold™ Kit, effecting the conversion of unmethylated cytosine (C) to uridine (U) while preserving the methylated cytosine state. Specific primers targeting CpG island sequences were generated using Primer3. High-throughput sequencing was performed on an Illumina Hiseq with a 2×150 bp paired-end configuration.

To ensure sequencing quality, FastQC was employed, facilitating the exclusion of low-quality reads. FLASH executed joint paired-end sequencing, establishing a minimum acceptable length of 15 bp and an error rate below 10%. BLAST + facilitated mapping to the human reference genome, defining a helpful sequence with a joint coverage fraction exceeding 90%. Principal component analysis (PCA) using the R prompt function enabled sample clustering based on sperm methylation patterns.

Real-time quantitative PCR (RT-qPCR)

We meticulously selected five sets of semen samples for the analysis of four genes, and used TRIzol reagent (Invitrogen, Carlsbad, CA92008 USA) to extract total RNA from semen samples of control and experimental groups. Subsequently, cDNA synthesis was performed using the PrimeScript RT reagent Kit (Takara, Shiga, Japan), followed by amplification with gene-specific primers and RT-qPCR analysis using the LightCycler 480 SYBR Green I Master (Roche). Primer details can be found in the Table S1. β-actin served as the internal reference for normalization. Data analysis was conducted using the $2-\Delta\Delta Ct$ method in GraphPad Prism to determine the mRNA expression levels of the four genes.

Statistical analyses

Continuous variables were expressed as means ± standard deviations, counting data were expressed as frequencies (percentages) and analyzed using the chi-square test. Continuous data with a normal distribution were analyzed using t-test assessments to ascertain differences in methylation at specific sites, regions, genes, and methylation haplotypes between cases and unaffected controls. Significance in DNA methylation differences was determined at a p-value < 0.05 (two-tailed).

Results

Clinical features

A comprehensive overview of demographic characteristics is provided in Table 1. The absence of significant differences observed in critical variables such as age, BMI, educational status, and residence between the two groups is noteworthy. Furthermore, an in-depth analysis of semen parameters, encompassing semen volume, sperm counts, progressive sperm malformation rate, DNA fragmentation index, and abstinence time, revealed no significant disparities between the studied cohorts.

DNA methylation profiles of paternally imprinted genes in sperm of HBsAg-positive and controls

The curated set of 28 paternally imprinted genes under scrutiny encompassed 84 CpG islands and 1501 CpG sequences, as detailed in Supplementary Dataset 3. Notably, GNAS featured the highest count of CpG islands [15], while CACNA1A, CUL7, DNMT1, and MOCS1 each bore a single CpG island. All identified CpG islands harbored more than 10 CpG sequences, with the CACNA1C_5 island exhibiting the highest count at 36.

Evaluation of sulfite conversion efficiency indicated comparable outcomes between the experimental and control groups, suggesting the absence of systemic errors (Figure S1). Furthermore, the quality of DNA methylation was deemed satisfactory based on quality value and coverage, as outlined in Supplementary Dataset 4. Comprehensive methylation values for CpG sites targeted CpG island regions and individual genes are documented in Supplementary Dataset 5.

Principal component analysis (PCA) grounded in DNA methylation values of the CpG sites did not reveal significant distinctions between the two groups (Figure S2). This suggests a degree of homogeneity in DNA methylation patterns across the majority of promoter regions associated with the paternally imprinted genes.

Bioinformatic analyses of differential sperm DNA methylation between HBsAg positive and controls

A meticulous evaluation of methylation values at specific sites targeted CpG island regions and genes were conducted to delineate differential DNA methylation patterns.

In each site's initial DNA methylation analysis, 42 significantly different methylation sites were identified across 29 CpG islands within 19 genes (Supplementary Dataset 6). Notably, eight genes displayed only one significantly different CpG site, while three genes harbored two such sites. Furthermore, four genes contained three significantly different CpG sites, and four more exhibited four significantly different CpG sites. The analysis of DNA methylation at each CpG island revealed significant differences in four islands, including CUL7_10, PRKAG2_56, DNMT1_11, and TP53_83 (Fig. 2, Supplementary Dataset 7). Among these, CUL7_10, PRKAG2_56, and TP53_83 exhibited significantly lower DNA methylation, while DNMT1_11 demonstrated significantly higher DNA methylation in HBsAg-positive patients compared to controls. These differences translated into substantial variations within the CpG methylation islands, indicating region-specific methylation alterations (Fig. 2).

Table 1 Comparison of demographic characteristics between HBsAg positive and negative subjects

	Controls (*N* = 18)	Cases (*N* = 17)	t/χ^2	*P*
Age	31.72 ± 4.24	33.65 ± 2.91	1.556	0.129
BMI	25.20 ± 2.36	24.46 ± 2.90	0.829	0.413
Educational Status			0.238	0.625
Middle School	8	7		
University	9	11		
Residence			0..02	0.877
Rural	8	8		
Urban	9	10		
Semen volume(ml)	3.16 ± 0.79	2.92 ± 0.86	0.854	0.399
Sperm concentration(× 10^6/ml)	77.63 ± 33.20	78.70 ± 57.88	0.068	0.946
Progressive rate(PR%)	49.58 ± 12.80	44.10 ± 11.28	1.341	0.189
Sperm malformation rate(%)	94.72 ± 1.02	95.92 ± 0.85	1.799	0.081
DFI(%)	13.10 ± 5.85	16.87 ± 9.07	1.467	0.152
Abstinence time(days)	2.50 ± 0.51	2.41 ± 0.62	0.460	0.649

Chi-squared, or t -tests was used as appropriate. *DFI* DNA fragmentation index

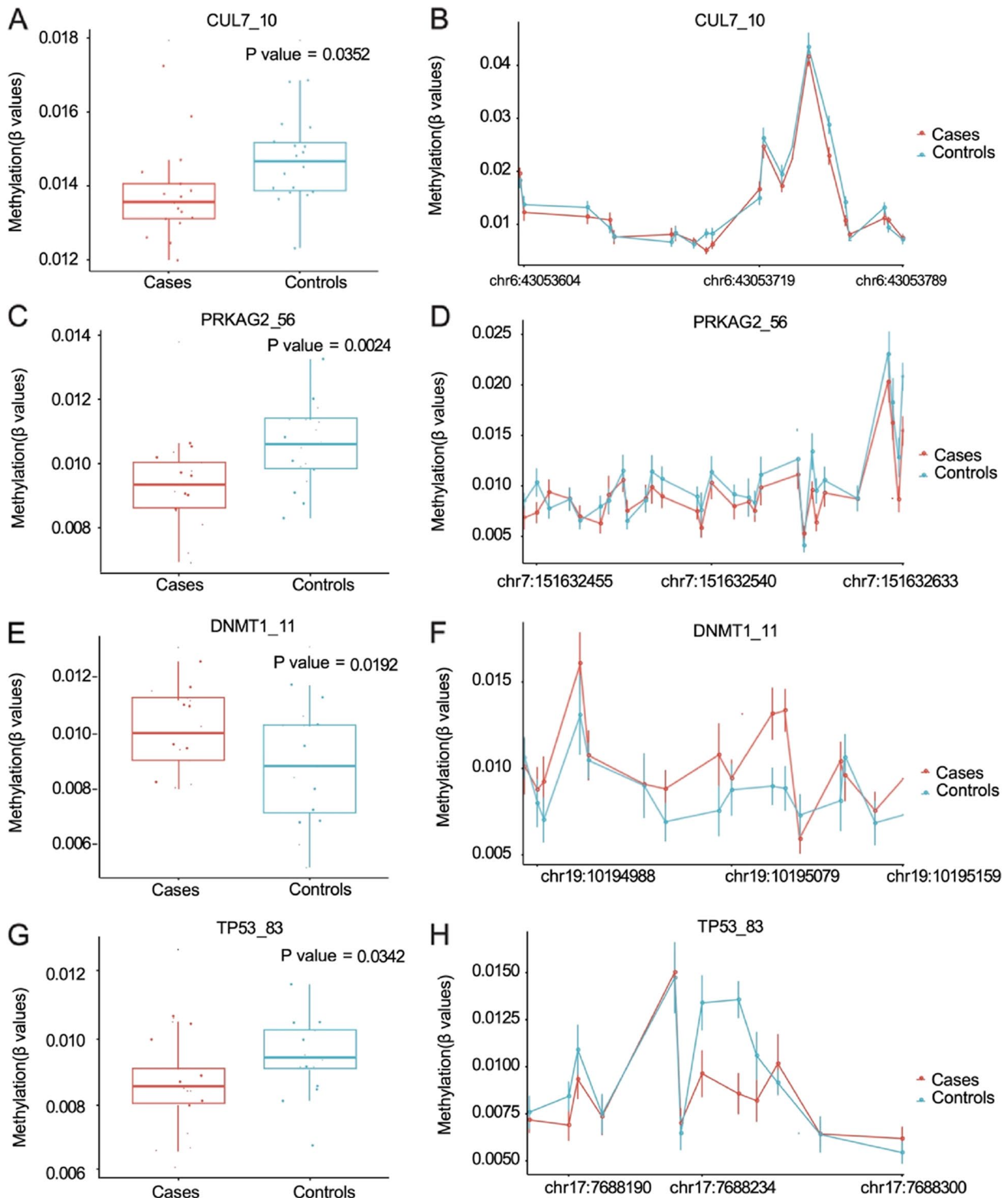

Fig. 2 Different methylation of targeted regions. Panels **A**, **C**, **E**, and **G** illustrated significant methylation in four targeted regions (CUL7_10, PRKAG2_56, DNMT1_11, TP53_83). Panels **B**, **D**, **F**, and **H** provided detailed methylation information for CpG sites in these regions

At the gene level, significant differences were observed in *CUL7*, *PRKAG2*, *DNMT1*, and *TP53*, corroborating findings from the targeted CpG island regions (Fig. 3, Supplementary Dataset 8). In order to explore the impact of methylation on the genes, we further conducted Real-time quantitative PCR (RT-qPCR) and found that the methylation can regulate the expression of gene. DNMT1 case group exhibited significantly decreased expression (increased methylation), and PRKAG2 and TP53 exhibited significantly increased expression relative to the control group (decreased methylation) (Fig. 3). These results suggest a potential modulation of paternal gene expression in offspring born to HBsAg-positive patients.

Conducting a DNA methylation haplotype analysis on the 28 imprinted genes (Supplementary Dataset 9) unveiled 51 significantly different DNA methylation haplotypes within 36 CpG islands across 22 genes (Fig. 4). Notably, *PRKAG2* and *GNAS* harbored six and four

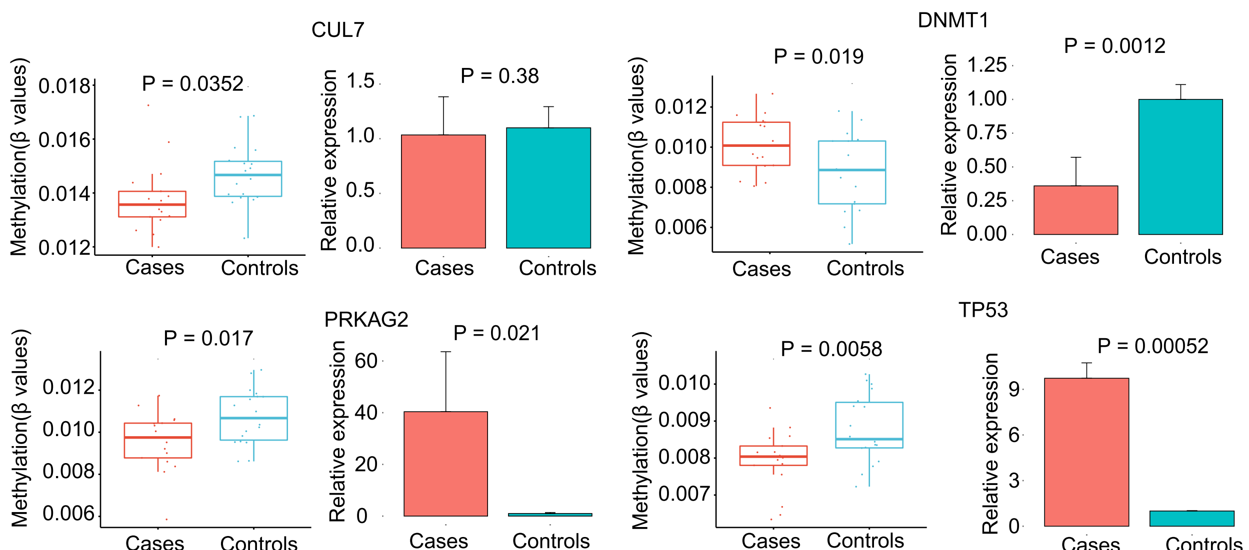

Fig. 3 Differential methylation and gene expression in genes. The results revealed statistically significant differences in methylation ($P < 0.05$) in four genes

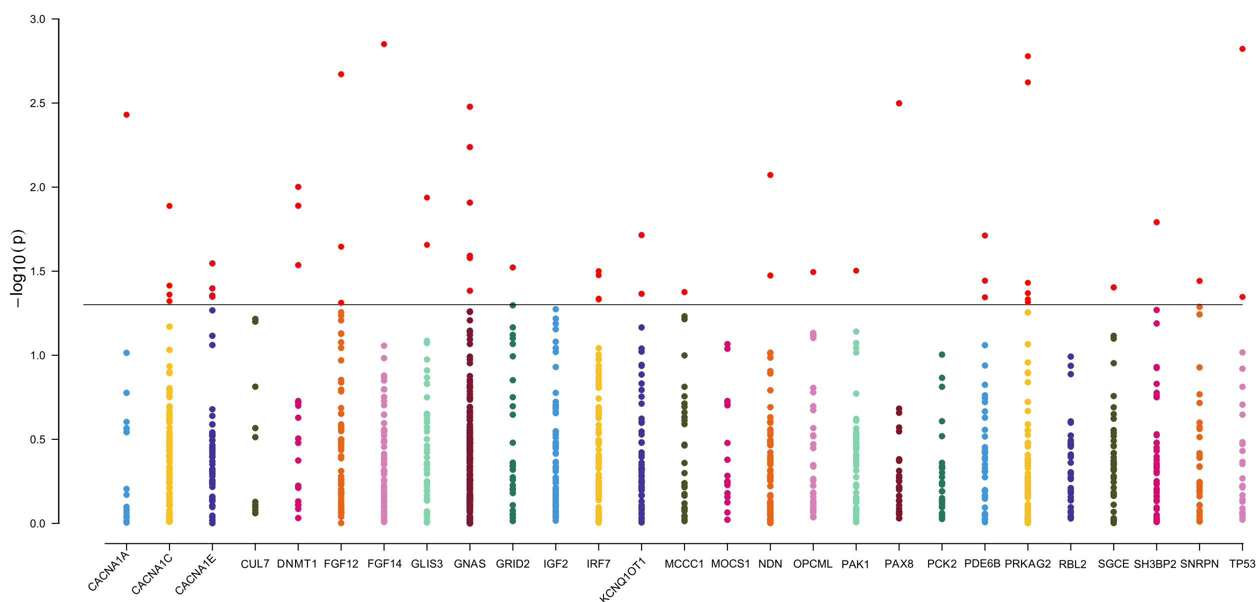

Fig. 4 Differential methylation haplotypes. The line in the figure indicated $P = 0.05$, and the dots above denoted significant methylation haplotypes with $P < 0.05$

significantly different DNA methylation haplotypes, respectively, while *CACNA1C*, *CACNA1E*, and *IRF7* contained four such haplotypes.

These findings illuminate the complex landscape of differential DNA methylation within imprinted genes associated with HBsAg-positive individuals, suggesting potential implications for offspring health.

Discussion

This is the first study to explore the uncharted territory of understanding how Hepatitis B Virus (HBV) infection influences DNA methylation in sperm. The comprehensive analysis unveiled 42 differentially methylated sites spread across 29 CpG islands within 19 genes. At the gene level, distinct patterns emerged, revealing an augmentation in methylation of *DNMT1* and a concomitant reduction in CUL7, PRKAG2, and TP53 methylation. The exploration also extended to a DNA methylation haplotype analysis, uncovering 51 differentially methylated haplotypes within 36 CpG islands across 22 genes. These intricate findings offer a glimpse into the potential mechanisms underpinning the intergenerational impact of paternal HBV infection. Identifying specific methylation alterations within essential genes adds a layer of understanding to the complex interplay between HBV infection and epigenetic modifications, shedding light on the intricate processes influencing paternal transmission effects across generations.

Epigenetics, the heritable regulation of gene expression through non-DNA encoded mechanisms, is a pivotal factor influencing cellular behavior [22, 23]. While previous studies have unequivocally demonstrated the correlation between Hepatitis B Virus (HBV) infection and adverse effects on sperm, including decreased motility and heightened rates of apoptosis and necrosis leading to diminished fertility [24, 25], the influence of HBV on sperm epigenetic information has remained unexplored until now. Our focus on DNA methylation, a fundamental mechanism of epigenetic inheritance, unravels intriguing insights into the potential impact of HBV on the epigenetic landscape of paternally imprinted genes in sperm.

DNA methylation of cytosine residues has been suggested to mediate parental effects in mammals [26]. Genomic imprinting, wherein gene expression depends on parental inheritance, is linked to differences in DNA methylation states that can be transmitted across generations in mammals [27]. Despite parental HBV infection being identified as a risk factor for hepatoblastoma in children [16], the underlying mechanisms have remained elusive. Our study suggests that HBV infection induces alterations in DNA methylation patterns, specifically within disease-causing paternally imprinted genes in sperm [28]. This intriguing discovery implies that such changes may be transmitted to offspring, potentially influencing their health and development. Notably, among the differentially methylated genes, TP53, encoding a well-known tumor suppressor, emerges as a candidate influencing cancer incidence in the offspring [29], paving the way for further investigations into tumorigenesis in children born to HBV-infected male patients.

While methylation is predominantly erased upon fertilization in mammals [30], the impact of alterations in this process on gene expression in progeny remains unclear. Evidence supporting the possibility of imprinted genes escaping erasure processes and passing on their DNA methylation status to progeny is known [31]. Our study systematically screened for paternally imprinted genes represented in disease-causing genes listed in the OMIM database, targeting their promoter regions for DNA methylation detection. Accurate quantification of methylation levels in target genes, such as CUL7, PRKAG2, DNMT1, and TP53, offers insight into potential cascading effects stemming from alterations in the expression patterns of these proteins. This has significant implications for the health of future generations. Notably, CUL7, recognized as a modifier in ubiquitination processes [32], and PRKAG2, involved in phosphorylation modifications, may additionally govern the function of multiple proteins during post-translational modifications [33]. Furthermore, DNMT1, a DNA methyltransferase, has the capacity to regulate DNA methylation in embryos and offspring [34].

Acknowledging the limitations of a small sample size that may have influenced result accuracy due to random factors, our study focuses solely on promoter DNA methylation without analyzing other epigenetic alterations associated with HBV infection. Future endeavors within our group aim to address these limitations by investigating larger sample groups to gain a more comprehensive understanding of the additional effects exerted by HBV infection in sperm.

Conclusion

In conclusion, our study identifies significant differences in promoter DNA methylation levels within several paternally imprinted genes between HBV-positive patients and control individuals. As the first to shed light on the effects of HBV infection on sperm DNA methylation, our findings provide valuable clues to the transmission mechanisms responsible for the intergenerational effects of HBV, opening avenues for further research into potential health-related consequences in offspring.

Competing of interests

The authors declare that they have no competing interests.

Abbreviations

HBV Hepatitis B virus
ART Assisted reproductive technology
HBsAg Hepatitis B surface antigen
PCA Principal component analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12860-024-00515-7.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

Supplementary Material 6.

Supplementary Material 7.

Supplementary Material 8.

Supplementary Material 9.

Supplementary Material 10.

Supplementary Material 11: Figure S1. Assessment of **s**ulfite conversion efficiency. The result indicated comparable outcomes between the experimental and control groups, suggesting the absence of systemic errors.

Supplementary Material 12: Figure S2. Principal component analysis. PCA based on DNA methylation values of CpG sites did not reveal significant distinctions between the experimental and control groups.

Acknowledgements

We want to thank all the individuals who participated in and supported this research.

Authors' contributions

KL, RH, ML and DT designed the study. BW, YS, ML, LR, HG, WY, CW and CX collected the samples and data. KL and DT performed the data analysis. KL, ML and WY wrote the paper. All authors have read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (No. 82101681 and 82101944), Research Fund of Anhui Institute of translational medicine (2023zhyx-C26 and 2023zhyx-C58).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request, without undue reservation. The datasets generated and/or analysed during the current study are available in the NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE270937 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc =GSE270937).

Declarations

Ethics approval and consent to participate

The local research ethics committee of the First Affiliated Hospital of Anhui Medical University approved this study. Informed consent was obtained from all subjects. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing of interests

The authors declare that they have no competing interests.

Received: 19 December 2023 Accepted: 24 July 2024
Published online: 01 August 2024

References

1. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014;384(9959):2053–63.
2. Hatzakis A, Van Damme P, Alcorn K, Gore C, Benazzouz M, Berkane S, et al. The state of hepatitis B and C in the Mediterranean and Balkan countries: report from a summit conference. J Viral Hepat. 2013;20(Suppl 2):1–20.
3. Ruan LL, Chen MX, Adu-Gyamfi EA, Geng LH, Fu LJ, Wan Q, et al. Maternal Hepatitis B Virus Infection and Pregnancy Outcomes of Freeze-Thaw Embryo Transfer. JAMA Netw Open. 2023;6(7): e2323495.
4. Cui AM, Cheng XY, Shao JG, Li HB, Wang XL, Shen Y, et al. Maternal hepatitis B virus carrier status and pregnancy outcomes: a prospective cohort study. BMC Pregnancy Childbirth. 2016;16:87.
5. Zheng S, Zhang H, Chen R, Yan J, Han Q. Pregnancy complicated with hepatitis B virus infection and preterm birth: a retrospective cohort study. BMC Pregnancy Childbirth. 2021;21(1):513.
6. Nguyen MH, Wong G, Gane E, Kao JH, Dusheiko G. Hepatitis B virus: advances in prevention, diagnosis, and therapy. Clin Microbiol Rev. 2020;33(2):e00046–19.
7. Rehermann B. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nat Med. 2013;19(7):859–68.
8. Hann HW, Hann RS, Maddrey WC. Hepatitis B virus infection in 6,130 unvaccinated Korean-Americans surveyed between 1988 and 1990. Am J Gastroenterol. 2007;102(4):767–72.
9. Hadchouel M, Scotto J, Huret JL, Molinie C, Villa E, Degos F, et al. Presence of HBV DNA in spermatozoa: a possible vertical transmission of HBV via the germ line. J Med Virol. 1985;16(1):61–6.
10. Huang JM, Huang TH, Qiu HY, Fang XW, Zhuang TG, Liu HX, et al. Effects of hepatitis B virus infection on human sperm chromosomes. World J Gastroenterol. 2003;9(4):736–40.
11. Huang JM, Huang TH, Qiu HY, Fang XW, Zhuang TG, Qiu JW. Studies on the integration of hepatitis B virus DNA sequence in human sperm chromosomes. Asian J Androl. 2002;4(3):209–12.
12. Wang S, Peng G, Li M, Xiao H, Jiang P, Zeng N, et al. Identification of hepatitis B virus vertical transmission from father to fetus by direct sequencing. Southeast Asian J Trop Med Public Health. 2003;34(1):106–13.
13. Cai QX, Zhu YY. Is hepatitis B virus transmitted via the male germ line? A seroepidemiological study in fetuses. Int J Infect Dis. 2013;17(1):e54–8.
14. Xiong Y, Liu C, Wei W, Huang S, Wang J, Qi Y, et al. The impact of biparental hepatitis B virus infection on pregnancy outcomes in patients undergoing assisted reproductive technology treatment: a systematic review and meta-analysis. Arch Gynecol Obstet. 2022;306(4):1253–66.
15. Lorusso F, Palmisano M, Chironna M, Vacca M, Masciandaro P, Bassi E, et al. Impact of chronic viral diseases on semen parameters. Andrologia. 2010;42(2):121–6.
16. Heck JE, Wu CK, Huang X, Chew KW, Tong M, Federman N, et al. Cohort study of familial viral hepatitis and risks of paediatric cancers. Int J Epidemiol. 2022;51(2):448–57.
17. Zhong C, Lu H, Han T, Tan X, Li P, Huang J, et al. CpG methylation participates in regulation of hepatitis B virus gene expression in host sperm and sperm-derived embryos. Epigenomics. 2017;9(2):123–5.
18. Koumbi L, Karayiannis P. The Epigenetic Control of Hepatitis B Virus Modulates the Outcome of Infection. Front Microbiol. 2015;6:1491.
19. Cheng Q, Zhao B, Huang Z, Su Y, Chen B, Yang S, et al. Epigenome-wide study for the offspring exposed to maternal HBV infection during pregnancy, a pilot study. Gene. 2018;658:76–85.
20. World Health Organization: WHO Laboratory Manual for the Examination and Processing of Human Semen, 6th ed..https://www.who.int/publications/i/item/9789240030787 (2021). Accessed 17 Nov 2023.

21. Tucci V, Isles AR, Kelsey G, Ferguson-Smith AC. Genomic Imprinting and Physiological Processes in Mammals. Cell. 2019;176(5):952–65.
22. Rotondo JC, Borghi A, Selvatici R, Magri E, Bianchini E, Montinari E, et al. Hypermethylation-Induced Inactivation of the IRF6 Gene as a Possible Early Event in Progression of Vulvar Squamous Cell Carcinoma Associated With Lichen Sclerosus. JAMA Dermatol. 2016;152(8):928–33.
23. Keyhan S, Burke E, Schrott R, Huang Z, Grenier C, Price T, et al. Male obesity impacts DNA methylation reprogramming in sperm. Clin Epigenetics. 2021;13(1):17.
24. Moretti E, Federico MG, Giannerini V, Collodel G. Sperm ultrastructure and meiotic segregation in a group of patients with chronic hepatitis B and C. Andrologia. 2008;40(5):286–91.
25. Zhou XL, Sun PN, Huang TH, Xie QD, Kang XJ, Liu LM. Effects of hepatitis B virus S protein on human sperm function. Hum Reprod. 2009;24(7):1575–83.
26. Rando OJ, Simmons RA. I'm eating for two: parental dietary effects on offspring metabolism. Cell. 2015;161(1):93–105.
27. Peters J. The role of genomic imprinting in biology and disease: an expanding view. Nat Rev Genet. 2014;15(8):517–30.
28. Rotondo JC, Lanzillotti C, Mazziotta C, Tognon M, Martini F. Epigenetics of Male Infertility: The Role of DNA Methylation. Front Cell Dev Biol. 2021;9: 689624.
29. Huang J, Sengupta R, Espejo AB, Lee MG, Dorsey JA, Richter M, et al. p53 is regulated by the lysine demethylase LSD1. Nature. 2007;449(7158):105–8.
30. Feng S, Jacobsen SE, Reik W. Epigenetic reprogramming in plant and animal development. Science. 2010;330(6004):622–7.
31. Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, et al. Programming and inheritance of parental DNA methylomes in mammals. Cell. 2014;157(4):979–91.
32. Hopf LVM, Baek K, Klügel M, von Gronau S, Xiong Y, Schulman BA. Structure of CRL7(FBXW8) reveals coupling with CUL1-RBX1/ROC1 for multi-cullin-RING E3-catalyzed ubiquitin ligation. Nat Struct Mol Biol. 2022;29(9):854–62.
33. Lopez-Sainz A, Dominguez F, Lopes LR, Ochoa JP, Barriales-Villa R, Climent V, et al. Clinical Features and Natural History of PRKAG2 Variant Cardiac Glycogenosis. J Am Coll Cardiol. 2020;76(2):186–97.
34. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005;74:481–514.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.