RESEARCH LETTER

Water Avoidance-Stress Induces Differential Colon Transcriptomic Responses in BALB/c and C57BL/6 Mice Irritable Bowel Syndrome Model

High-quality transcriptome data of irritable bowel syndrome (IBS) patient colonoscopy biopsies were generated, and animal models that mimic human disease transcriptomic changes could help explore the underlying pathogenesis mechanism. Chronic water avoidance stress (WAS) is one of the most reproduced animal models representing IBS; we found that hypothalamic-pituitary-adrenal axis-derived endogenous glucocorticoid (GC) impaired colon intestinal barrier function of WAS-induced IBS rats via glucocorticoid receptor's (GR/NR3C1) transcriptional regulation.[1,2] WAS elevated GC binds its nuclear receptor GR in the cytosol, and GR is translocated to the nucleus, where GR modulates the transcriptome as a transcription factor (TF) in the stress responses. We found that GR downregulates tight junction protein claudin 1 in WAS rats; this phenomenon was reproduced in BALB/c but not C57BL/6 mice.[2,3] The outbred rats demonstrated subpopulation-specific stress responses, and inbred prototypical Th1- (C57BL/6) and Th2-(BALB/c) type mouse strains are frequently compared for their stain-differential stress responses.[1,4–6] BALB/c mice had significantly greater accumulated corticosterone (GC in rodents) levels than C57BL/6 mice after chronic stress.[4] They had differential stress responses to affect behavior, colonic serotonin (5-hydroxytryptamine/5-HT) levels, defecation pattern, dysbiosis (disruption to the microbiota homeostasis), and risk of colitis.[3–6] We reproduced WAS-induced IBS phenotypes, including barrier dysfunction, inflammatory infiltration, and visceral hyperalgesia in BALB/c mice.[7] GR repressed *Nr1d1* at promoter E-boxes in response to stress and mimicked decreased *NR1D1* observed in IBS patients.[7] These E-boxes can also be occupied by clock circadian regulator: basic helix-loop-helix ARNT like 1 competitor upstream transcription factor 1, the *Usf1* promoter single nucleotide polymorphism may contribute to the strain-differential stress responses in addition to the circadian.[7,8] In C57BL/6J, we found that WAS decreased mucin 2 mucus secretion and intestinal motility.[9] Whether these strains can represent subtype-specific pathology in stress-induced IBS is intriguing.

In this study, we provided a high-quality dataset of murine WAS-induced IBS model colon epithelium transcriptome; differentially expressed genes (DEGs) in animal models mimicked some transcriptional changes observed in IBS patient colonoscopy biopsies (Table). Within the BART (Binding Analysis for Regulation of Transcription) analysis of those DEGs, GR/NR3C1 was the crucial transcriptional regulator in both BALB/c and C57BL/6 mice. However, GR downstream circadian TFs may dominate DEGs in BALB/c, and NF-Kappa B signaling TFs may dominate DEGs in C57BL/6, respectively (Figure C–E). Shared cohesin components structural maintenance of chromosomes 1, structural maintenance of chromosomes 3, and RAD21 supported the GR-NIPBL-cohesin mediated chromatin looping mechanism in stress-impaired intestinal homeostasis, consistent with our 4D nucleome study (Figure E).[7] Decreased *NR1D1* in IBS patients suggested an impaired circadian transcription network (Figure A1).[7] Genetically determined differential *Nr1d1* and *Dbp* transcription may participate in the strain-differential stress altered transcription.[7,8] In addition to IBS and inflammatory bowel disease DEGs encoded within the conserved *Nr1d1-Med24-Ormdl3-Ikzf3-Grb7-Erbb2-Stard3-Fbxl20* chromatin axis, validated chromatin 3D structure regulated circadian genes *Nr1d2*, *Npas2*, *Rorc*, *Pard3*, and *Rnf125* also exhibited BALB/c-specific stress responses (Figures D and A1).[7] In contrast to circadian DEGs observed in BALB/c, *Timeless* elevated in C57BL/6J (Figure A1). *IRF7* was identified as a significant Interferon Gamma Signaling DEG in a reanalysis of published IBS transcriptome data.[10] IBS patient fecal supernatants could significantly induce *IRF7* in colonoids monolayers, suggesting a potential role of IRF7 in IBS pathogenesis. Significant *Irf7* changes in C57BL/6 suggest the suitability in studying this mechanism (Figure A1). Signal transducer and activator of transcription 1 was predicted as one of the top regulatory TFs of IBS DEGs, recent animal studies suggest that signal transducer and activator of transcription 1 is involved in stress-induced visceral hypersensitivity and could serve as a therapeutic target.[10] Within GR knockout C57BL/6 mice, *Stat1* was identified as the crucial GR target gene responsible for phenotypes, including inflammation. The colitis-related GR target gene *Stat1* and *Stat2* (~18kb downstream *Timeless*) increased in C57BL/6J only, consistent with C57BL/6's vulnerability to prolonged WAS-induced colitis (Figure A1).[6] The IBS DEG homolog *Ifit1* and *Ifit3* had C57BL/6 specific changes; they are GR-STAT1 regulated interferon-stimulated genes responsible for intestinal inflammation. In addition to increased transcription within the *Ifit3-Ifit1* chromatin area in the C57BL/6, we noticed altered GR binding and chromatin 3D structures in BALB/c (Figure A3).[7] These data suggest the participation of the GR-modulated 4D nucleome in differential gut brain axis stress responses.[7] The GR target DEGs, including *Nr1d1*, *Nr1d2*, *Maoa* (Mono Amine Oxidase-A, catalyzes the degradation process of various amine neurotransmitters, including 5-HT), *Sgk1*, and *Fkbp5*, are also consistent with the homologous human GR regulome (Figure A1). The lower *Maoa* baseline level in C57BL/6J is consistent with a higher colonic 5-HT baseline. The DEGs *Maoa* and *Slc6a4* correlated with

Table. Overlapped Published IBS Patient Colonoscopy Biopsy DEGs With WAS-induced IBS Murine Model DEGs

Gene	Strain	WAS-insuced change	Human	Comparism	Significance	PMID
					Fold change	
Nr1d1	BALB/c	↓	*NR1D1*	Healthy control vs all IBS	↓1.44 $P = .0008$	22684480
					Log2-fold change	
Ikzf3	C57BL/6J	↑	*IKZF3*	IBS-D vs IBS-C left colon	↑*1.411* $P = 9.87E-06$	35502856
			IKZF3	IBS-D vs IBS-C right colon	↑*1.414* $P = 2.17E-08$	35502856
			IKZF3	IBS-D vs H right colon	↑1.082 $P = .002777$	35502856
			IKZF3	IBS-D vs H left colon	↑1.277 $P = 7.66E-05$	35502856
Fos	BALB/c	↓	*FOS*	IBS-D vs H right colon	↑1.367 $P = .000306$	35502856
Nr4a2	C57BL/6J	↓	*NR4A2*	IBS-D vs H left colon	↑1.012 $P = .000671$	35502856
Pnp	C57BL/6J	↑	*PNP*	IBS-C vs H	↑0.499 $P = .000602$	32916129
			PNP	IBS-D vs H	↑0.65 $P = 2.02E-05$	32916129
Xdh	C57BL/6J	↑	*XDH*	IBS-C vs H	↑0.255 $P = .0271$	32916129
Cdh3	BALB/c	↓	*CDH3*	IBS-C vs H	↑1.09 $P = .0000302$	32916129
			CDH3	IBS-C vs IBS-D	↓-1.4 $P = .000126$	32916129
P2ry4	C57BL/6J	↑	*P2RY4*	IBS-D vs H	↑2.53 $P = 7.03E-08$	24763552
Guca2b	C57BL/6J	↓	*GUCA2B*	IBS-D vs H	↑1.10 $P = 1.15E-05$	24763552
Pdzd3	C57BL/6J	↑	*PDZD3*	IBS-D vs H	↑1.13 $P = 3.47E-05$	24763552
Aldoc	C57BL/6J	↓	*ALDOC*	IBS-D vs H	↑1.06 $P = 1.41E-05$	24763552
Abca1	BALB/c	↑	*ABCA1*	IBS-D vs H	↓-0.87 $P = 7.62E-05$	24763552
Ifit3	C57BL/6J	↑	*IFIT3*	IBS-D vs H	↓-1.69 $P = 8.25E-06$	24763552
Ifit1	C57BL/6J	↑	*IFIT1*	IBS-D vs H	↓-1.48 $P = .000173$	24763552
Mx1	C57BL/6J	↑	*MX1*	IBS-D vs H	↓-1.48 $P = 4.67E-05$	24763552
Oas2	C57BL/6J	↑	*OAS2*	IBS-D vs H	↓-1.29 $P = 8.73E-05$	24763552
Birc5	C57BL/6J	↑	*BIRC5*	IBS-D vs H	↑1.08 $P = 3.55E-05$	24763552
Gabbr1	C57BL/6J	↓	*GABBR1*	IBS-D vs IBS-C right colon	↑1.098 $P = .003$	35502856
Slc6a7	BALB/c	↑	*SLC6A7*	IBS-D vs IBS-C right colon	↑1.221 $P = .003$	35502856
			SLC6A7	IBS-D vs HT right colon	↑1.299 $P = 4.35E-03$	35502856
			SLC6A7	IBS-D vs HT left colon	↑1.054 $P = .001661$	35502856
Il21r	C57BL/6J	↑	*IL21R*	IBS-D vs IBS-C right colon	↑1.018 $P = 1.76E-05$	35502856
Tnfrsf25	C57BL/6J	↓	*TNFTSF25*	IBS-D vs H right colon	↑1.005 $P = .008753$	35502856
Hbegf	BALB/c	↓	*HBEGF*	IBS-D vs H right colon	↑1.020 $P = 8.58E-05$	35502856
			HBEGF	IBS-D vs H left colon	↑1.254 $P = .000223$	35502856
Tlcd2	C57BL/6J	↑	*TLCD2*	IBS-D vs H right colon	↑1.244 $P = 1.10E-06$	35502856
			TLCD2	IBS-D vs H left colon	↑1.081 $P = 5.07E-06$	35502856
Tlcd2	BALB/c	↓				
Soat2	C57BL/6J	↑	*SOAT2*	IBS-D vs H Right colon	↓-2.434 $P = .000235$	35502856
Atf3	C57BL/6J	↑	*ATF3*	IBS-C vs H left colon	↑1.234673 $P = .014649$	35502856
Trim40	C57BL/6J	↑	*TRIM40*	IBS vs H	↑1.443 $P = .000340765$	https://doi.org/10.1016/j.imu.2023.101241
Trim31	C57BL/6J	↑	*TRIM31*	IBS vs H	↓-1.087 $P = .029554229$	https://doi.org/10.1016/j.imu.2023.101242
Trim15	C57BL/6J	↑	*TRIM15*	IBS vs H	↑1.826 $P = .123763746$	https://doi.org/10.1016/j.imu.2023.101244
Psmb9	C57BL/6J	↑	*PSMB9*	IBS vs H	↑1.826 $P = .133152912$	https://doi.org/10.1016/j.imu.2023.101245
Irf7	C57BL/6J	↑	*IRF7*	IBS vs H	↑1.807 $P = .179422745$	https://doi.org/10.1016/j.imu.2023.101246
Notch4	C57BL/6J	↑	*NOTCH4*	IBS vs H	↑1.54 $P = .260487544$	https://doi.org/10.1016/j.imu.2023.101247
Tap1	C57BL/6J	↑	*TAP1*	IBS vs H	↑-1.842 $P = .346810994$	https://doi.org/10.1016/j.imu.2023.101248

PMID, PubMed unique identifier.

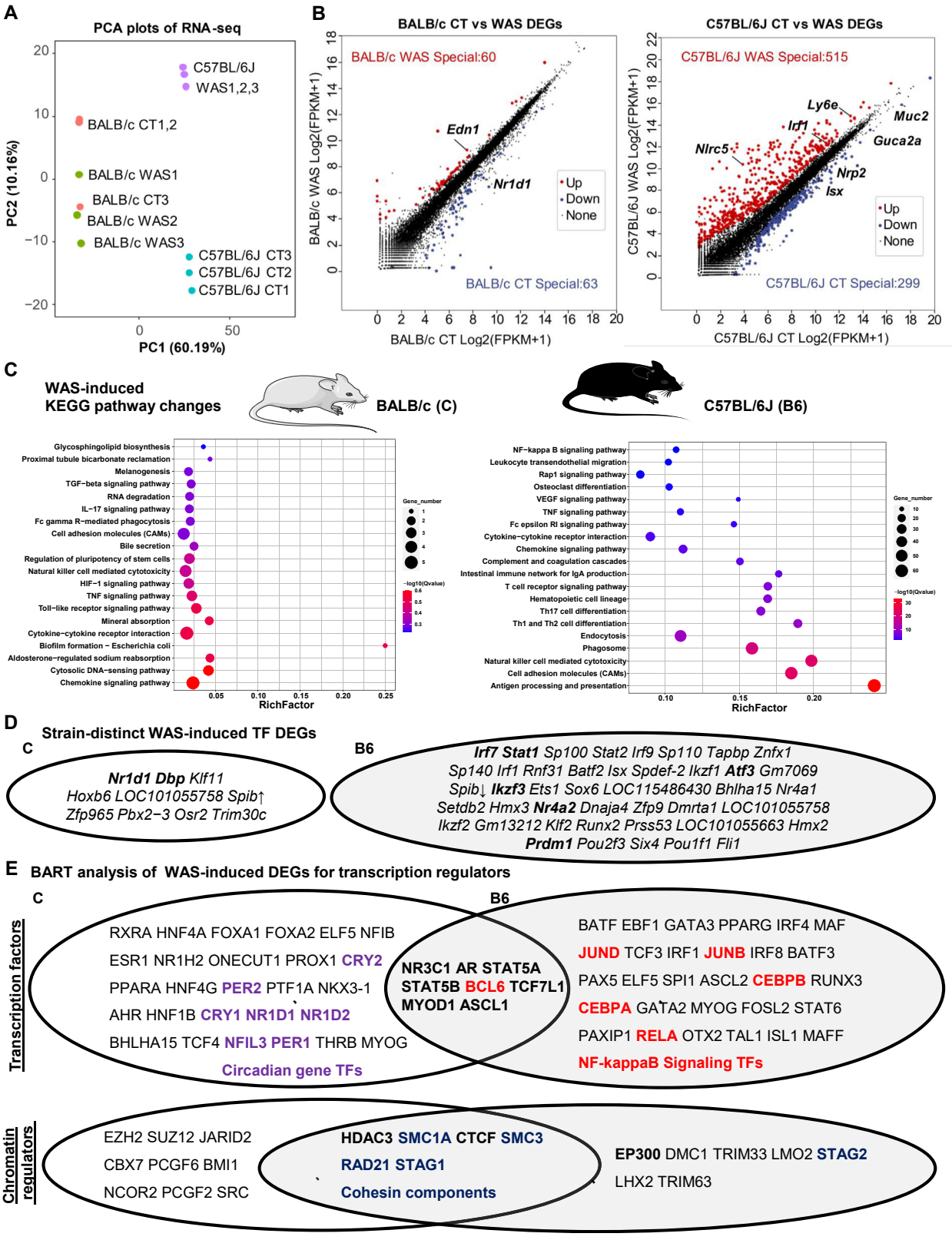

Figure. Strain-distinct stress-induced DEGs suggest circadian-stress crosstalk. Control (CT) and water avoidance stress (WAS) BALB/c (C) and C57BL/6J (B6) mouse colon epithelium cells were isolated for RNA-Seq analysis. (A) PC analysis. (B) Volcano plot analysis of DEGs. (C) KEGG pathway analysis of DEGs. (D) Transcription factor DEGs. (E) BART (Binding Analysis for Regulation of Transcription) analysis of WAS-induced DEGs for potential transcription regulators.

C57BL/6J specific stress-induced colonic 5-HT and defecation (Figure A1).[5] Potential IBS therapeutic targets *Slc9a3* (*hydrogen exchanger 3/Nhe3*) and *Nos2* (*Nitric Oxide Synthase 2/iNOS*) also demonstrated strain-distinct mRNA changes (Figure A1). DEGs hypothesized to be involved in IBS were also detected in mice, including *Ormdl3*, *Slc6a4* (*Sert/serotonin transporter*), *Gpbar1*, *Prdm1*, and *Cdh1* (*E-cadherin*) (Figure A1).

Epigenomic regulatory pathways played an essential role in IBS, and the effect of H3K9 modifications was verified in our animal studies.[1,9] We found that the C57BL/6 specific WAS-induced DEG *Muc2* was repressed by H3K9me3 in goblet cells (Figure B).[9] In the BART analysis of murine DEGs, histone deacetylase 3 was detected in both strains; however, histone acetyltransferase p300 (EP300) was detected in C57BL/6 only (Figure E). Histone deacetylase 3 and EP300 are responsible for H3K9 modification in the epigenetic mechanism of IBS, our dataset indicates strain differential epigenetic mechanisms between BALB/c and C57BL/6.[1,9] C57BL/6J strain is primarily used for translational purposes, ie, the inbred strain lacks the complexity observed in humans. BALB/c and C57BL/6J strains are extensively compared in multiple studies targeting the gut brain axis.[3,5,6] We first provided a side-by-side comparison between these trains in the IBS model and found almost distinct WAS-induced colon epithelium transcriptome responses, indicating the involvement of genetic background (for example *Usf1* single nucleotide polymorphism determining differential *Nr1d1* transcription) and epigenetic mechanisms (Figure).[7,8] The differential *Nr1d1* circadian gene transcription regulation between these strains indicates the involvement of the novel 4D-nucleome mechanism in determining differential stress responses between individuals.[7] This dataset can help to choose appropriate strains for specific mechanisms underlying IBS pathology and has potential applications for innovative therapeutic strategies in the precise treatment of human bowel disorders.[7,9] The BALB/c×C57BL/6J mouse model may help develop and validate next-generation pharmacogenomics and elucidate the role of allele-specific gene expression on genome architecture.[1,6,8]

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Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2024.07.016.

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2772-5723
https://doi.org/10.1016/j.gastha.2024.07.016

Received April 8, 2024. Accepted July 22, 2024.

Conflicts of Interest:
The authors disclose no conflicts.

Funding:
This study was supported in part by the National Natural Science Foundation of China (Nos. 82070556).

Ethical Statement:
All experimental procedures were performed following the ethical guidelines of the Animal Management Rules of the Chinese Ministry of Health (Document No.55,2001), and approved by the Animal Care and Use Committee, Union Hospital, Tongji Medical College, HUST, China (Approval ID 2016-0057).

Data Transparency Statement:
RNA-seq data can be accessed from NCBI accession PRJNA792732.

Reporting Guidelines:
ARRIVE/Care and Use of Laboratory Animals.