

Epigenetic Regulation in Chronic
Stress-induced Visceral Pain: The
Emerging Role of Intestinal Barrier
Dysfunction

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Introduction

Epigenetics refers to a variety of inheritable, potentially reversible processes that regulate gene activity and expression, independent of actual changes in DNA sequence (Misteli T, Cell, 2013).

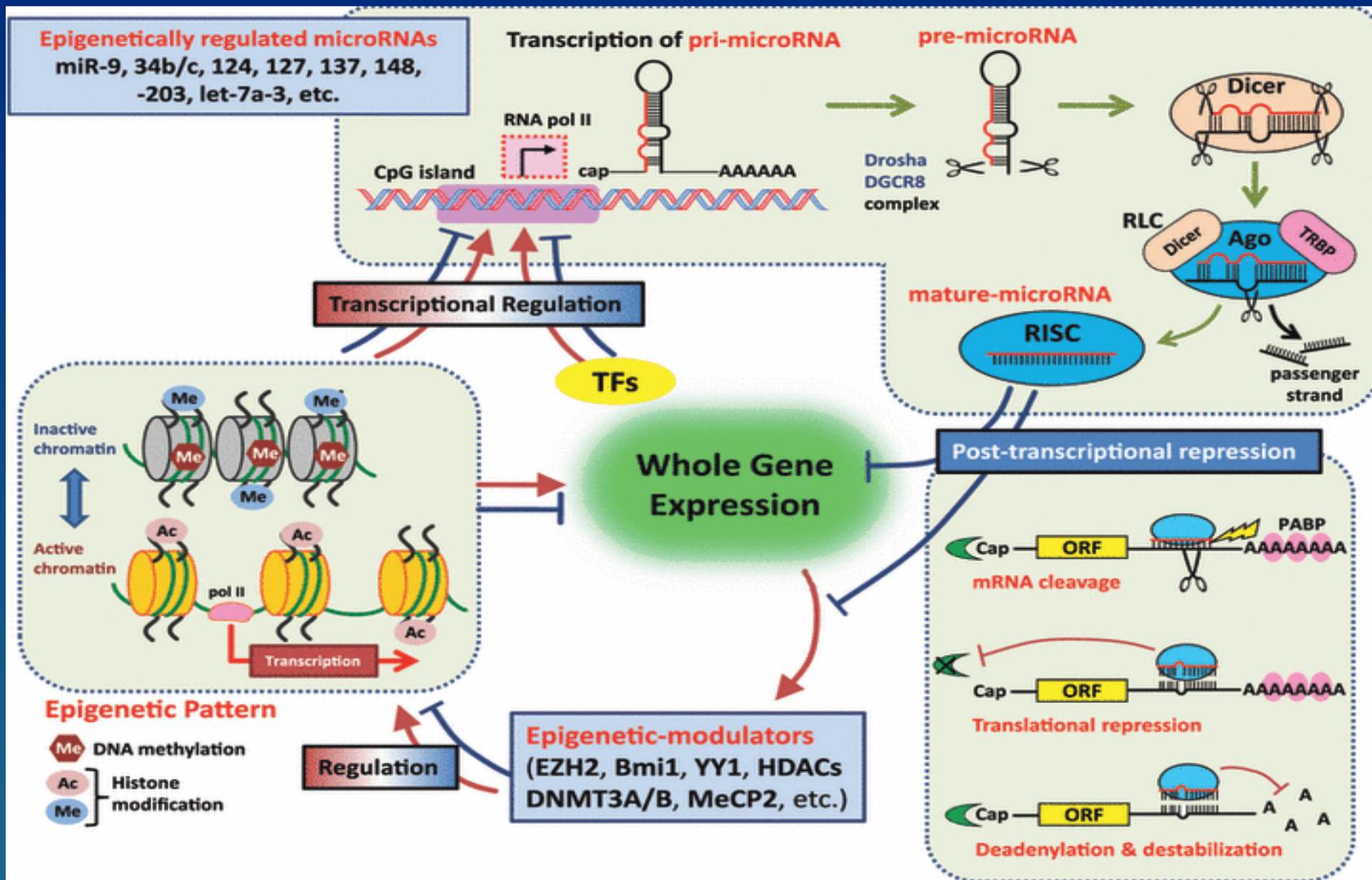
Mechanisms:

- DNA methylation by DNA methyltransferases (DNMTs). Typically inhibits gene transcription. Don't forget demethylases.
- Histone modification (Ex- histone acetyltransferases). Typically promotes gene transcription. Don't forget histone deacetylases.
- **microRNA regulation**. Typically inhibit gene expression.



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microRNA Regulation



Chronic Stress and Pain

Previously, we reported that chronic, intermittent stress-induced visceral hyperalgesia is associated with down-regulation of the anti-nociceptive endocannabinoid (CB1) receptor and up-regulation of the pro-nociceptive endovanilloid (TRPV1) receptor expression and function in primary afferent nociceptive (DRG) neurons (*Hong S et al., Gut, 2009; Hong S et al., Gastroenterology, 2011*).

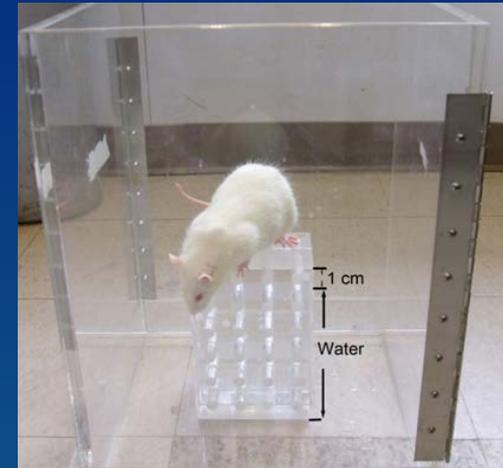
Hypothesis

DNA methyltransferases (DNMTs) and histone acetylation play an important role in the regulation of chronic stress-induced visceral hyperalgesia.

Methods

- **Animal model: chronic water avoidance (WA) stress**

Male rats were placed on the glass platform with water and maintained on the block for 1 hour daily for 10 consecutive days. The sham control rats were placed similarly for 1 hour daily for 10 days in the container without water. Some rats treated with GR antagonist RU-486 i.p., DNMT1 si-RNA or histone acetyltransferase, EP300 si-RNA injection at L6-S1 Q72hr during the stress phase.



- **Electromyographic (EMG) quantification**

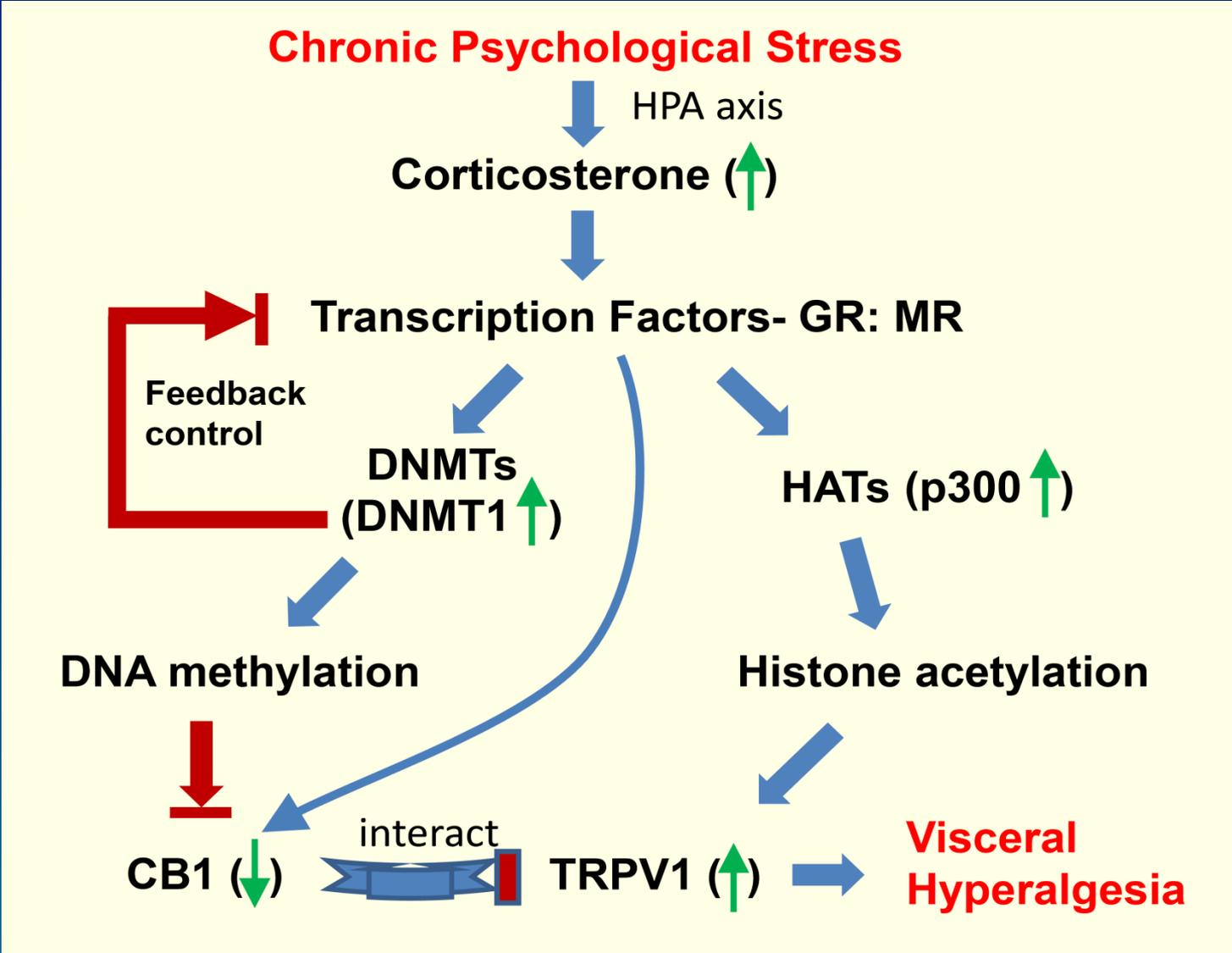
Visceral motor response (VMR) to colorectal distension (CRD) was used to measure visceral sensitivity.

- **Chromatin immunoprecipitation (ChIP)**

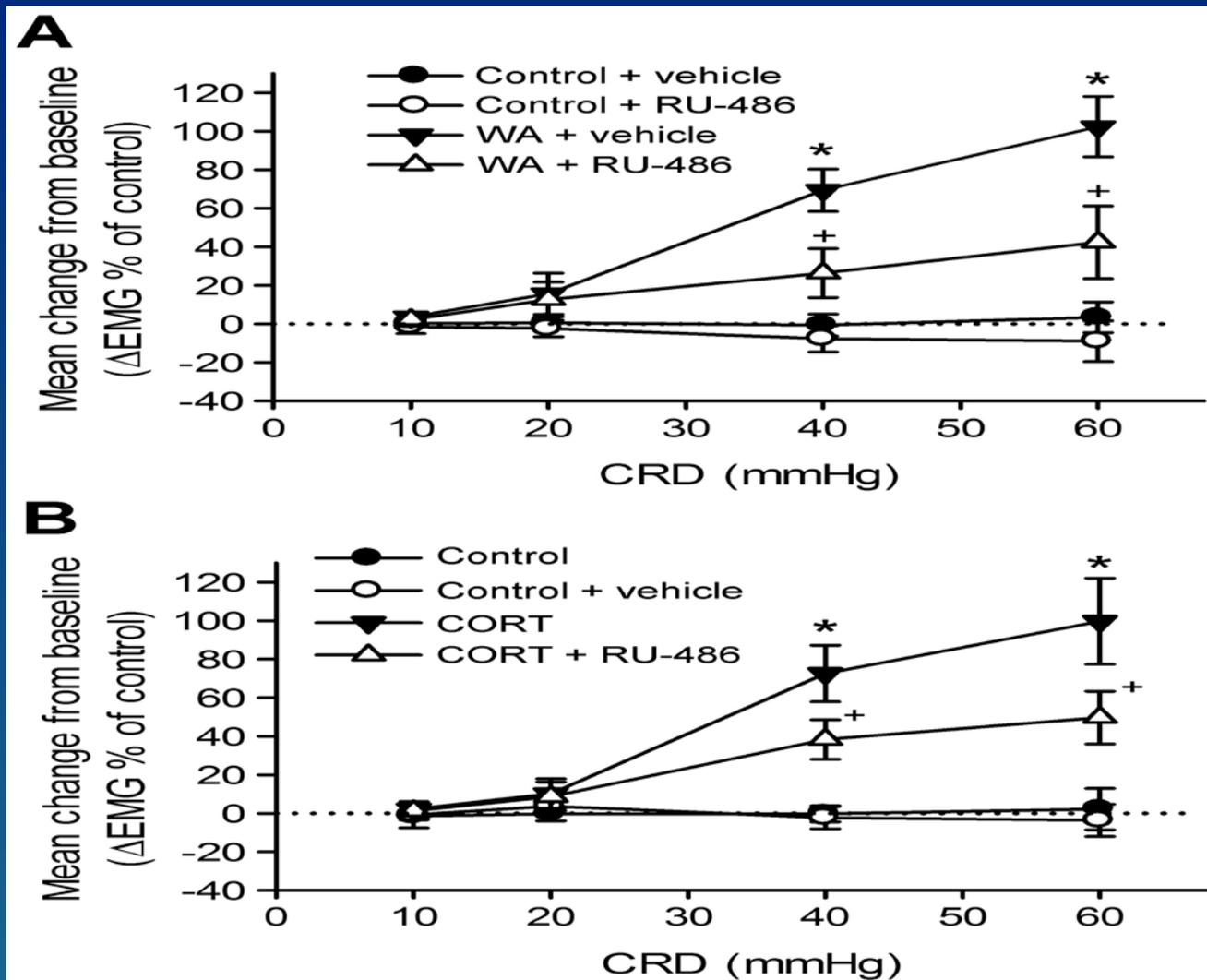
- **Methylated DNA immunoprecipitation and PCR (MeDIP)**

- **Pyrosequencing**

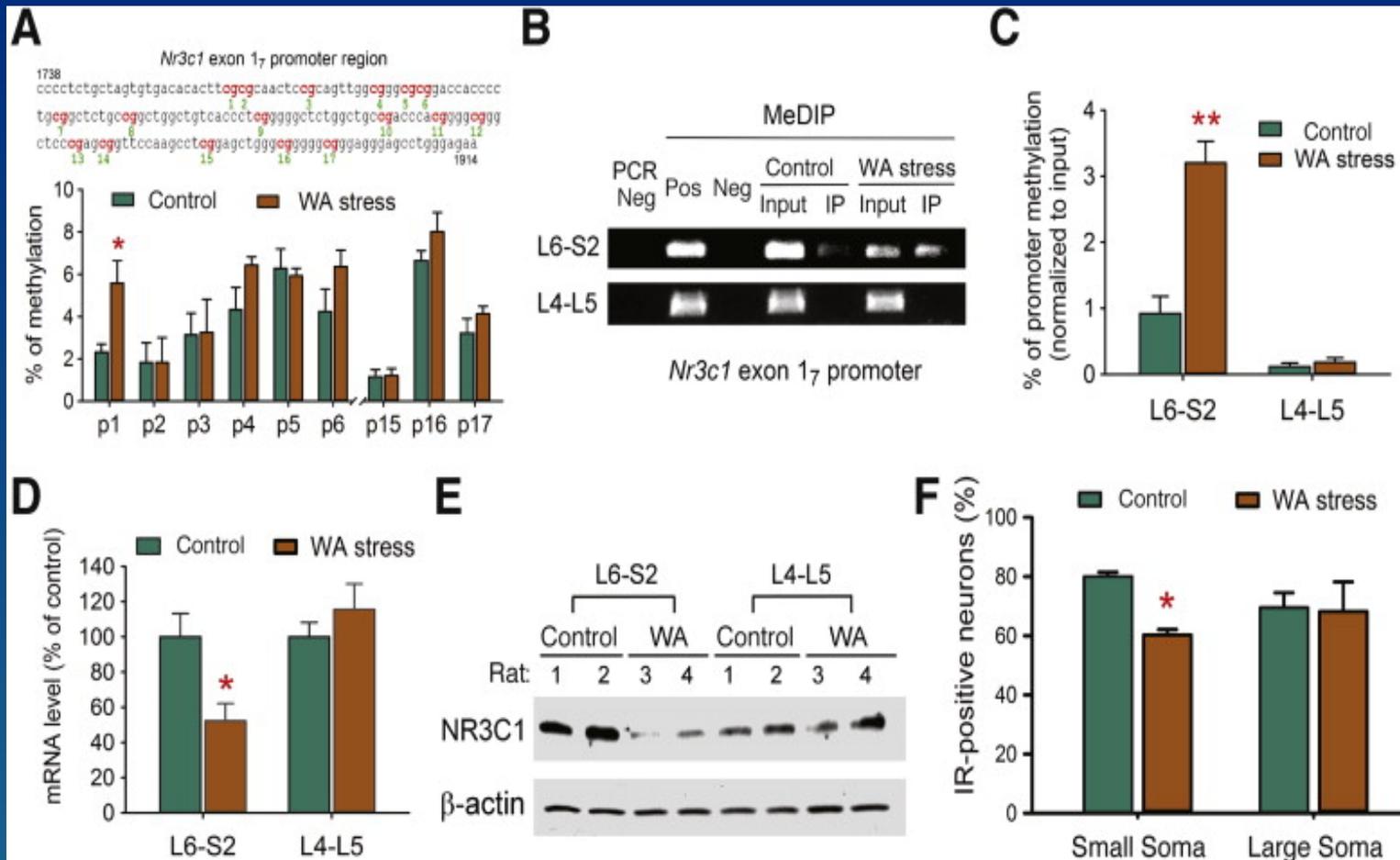
Epigenetic regulation of chronic stress-induced visceral hyperalgesia



Chronic Stress and Visceral Pain: Effect of GR Antagonist RU486 and CORT \pm RU486



Chronic WA stress induced region-specific DNA methylation at the *Nr3c1* (GR) promoter region and down-regulation in NR3C1 expression

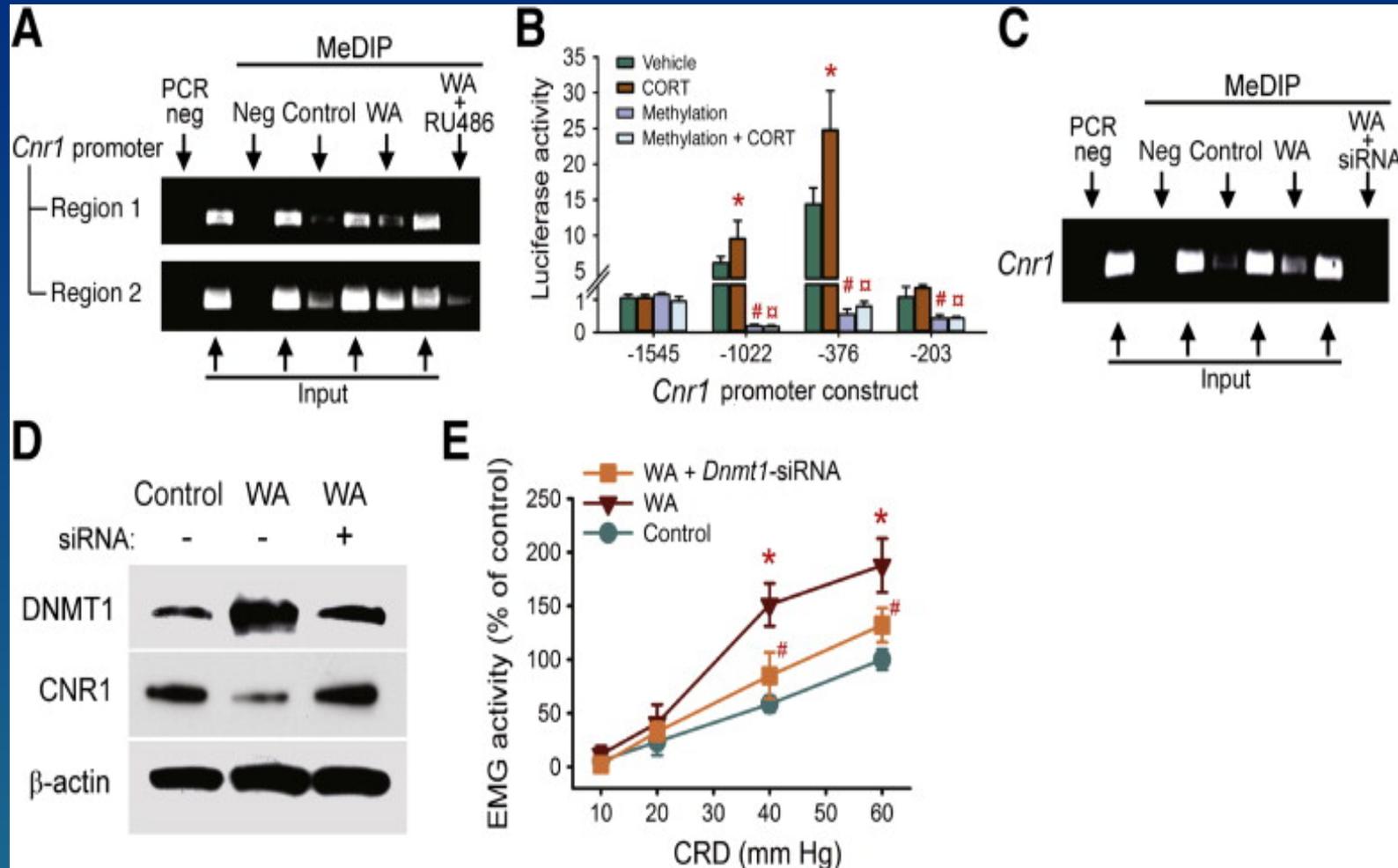


Shuangsong Hong , Gen Zheng , John W. Wiley

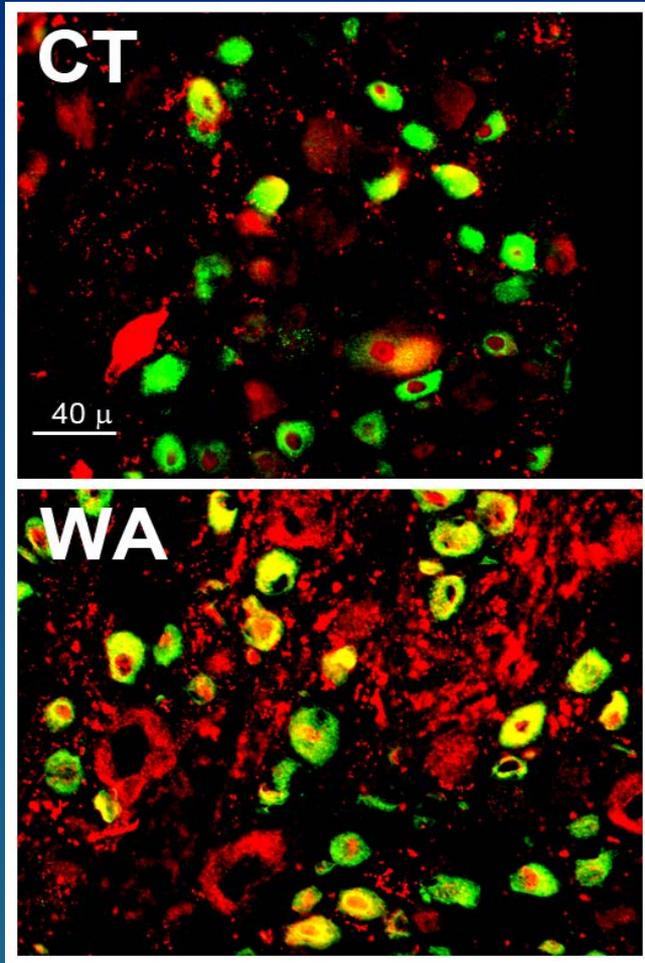
Epigenetic Regulation of Genes That Modulate Chronic Stress Induced Visceral Pain in the Peripheral Nervous System

Gastroenterology, Volume 148, Issue 1, 2015, 148 - 157

DNMT1 mediates *Cnr1* (CB1) promoter methylation and visceral pain perception in chronic stress

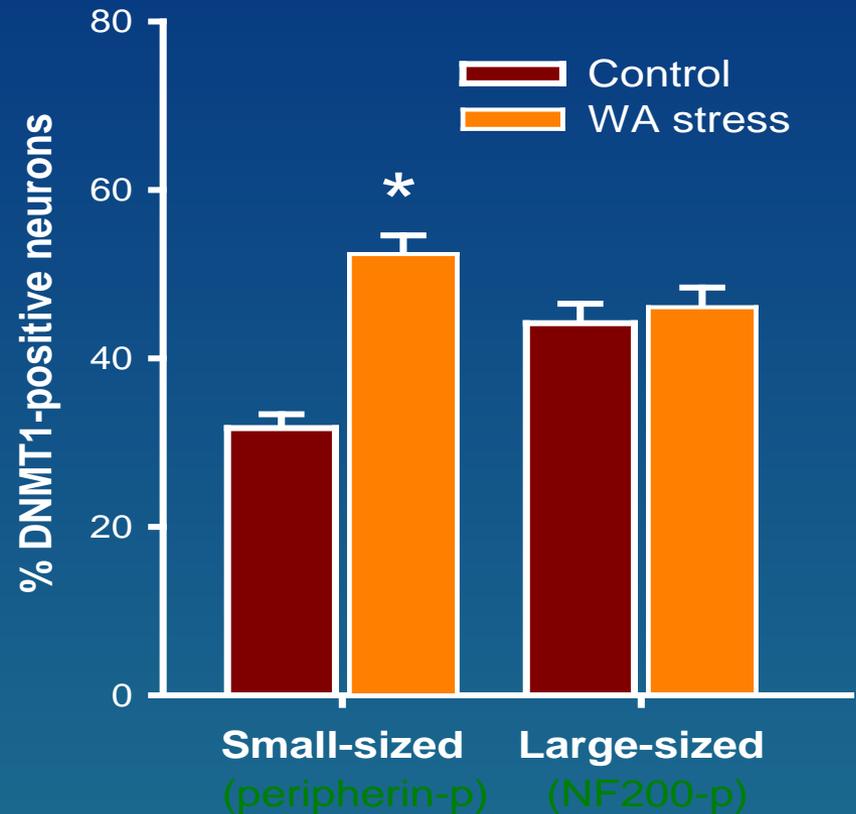


DNMT1 increased in small C-fiber neurons in L6-S2 DRGs in stress rats

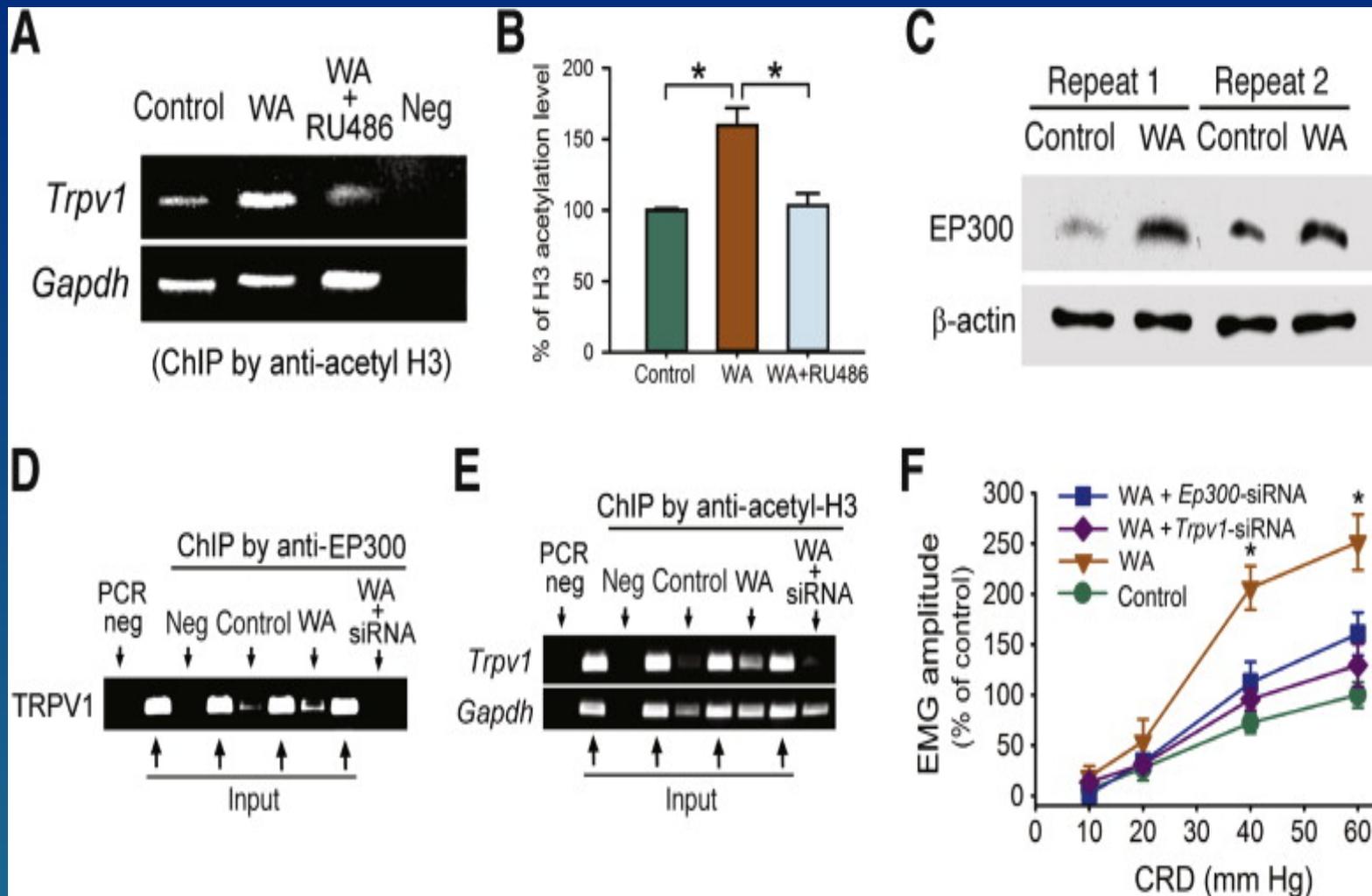


Red: DNMT1

Green: peripherin (C-fiber marker)



Histone acetyltransferase EP300 regulates *Trpv1* histone H3 acetylation, protein expression, and visceral pain perception in chronic stress

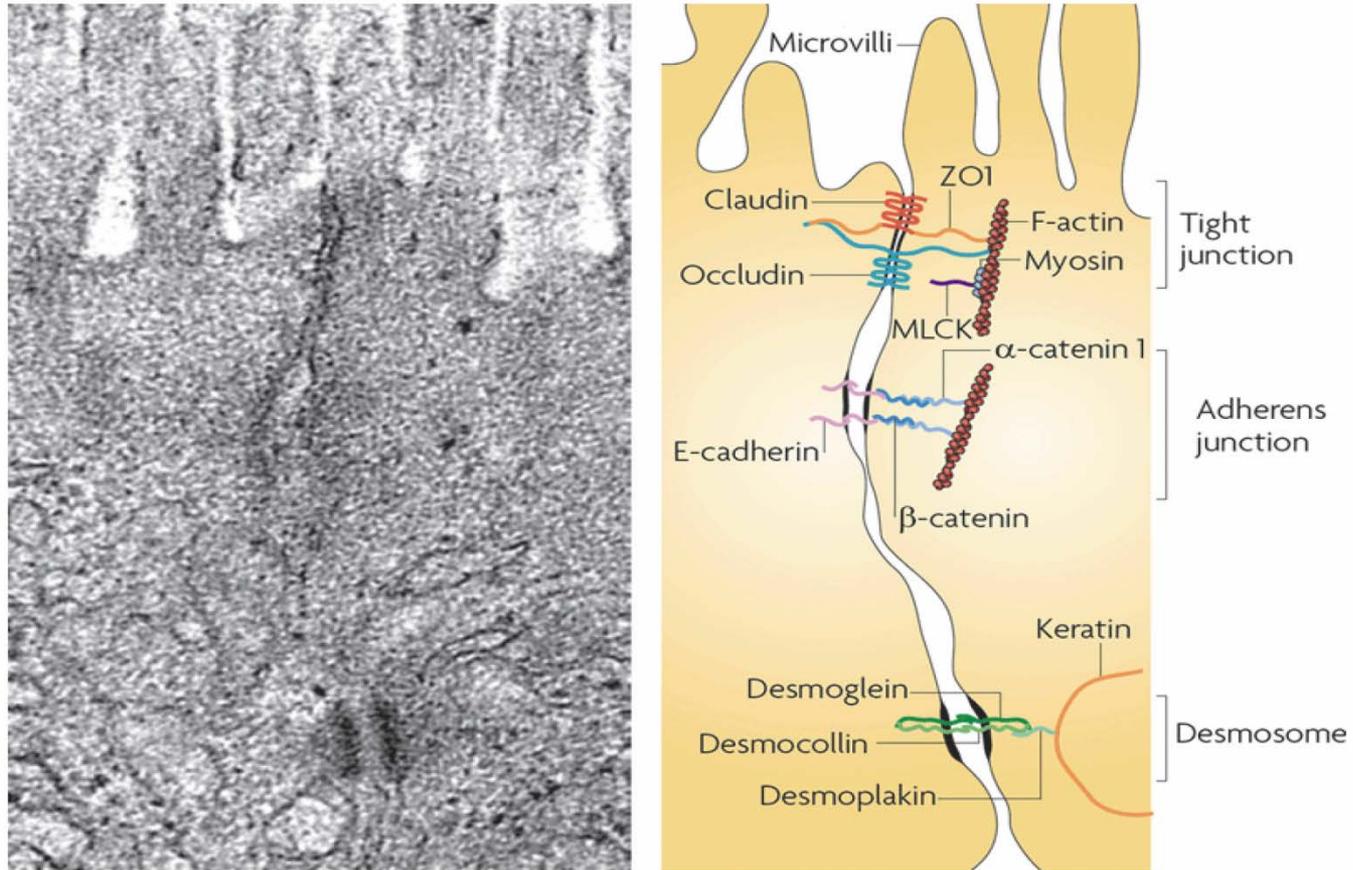


Chronic Stress: Effect on Epithelial Tight Junction Protein Expression and Function

Irritable Bowel Syndrome (IBS), Gut Barrier Permeability and Pain

- CRF treatment increased permeability to Horse Radish Peroxidase in human colon, blocked by mast cell stabilizer (Wallon C et al. Gut, 2008)
- Diarrhea-prone Irritable Bowel Syndrome (IBS) associated with increased permeability (measured by Lactose:Mannitol ratio) that correlated with increased pain (Zhou Q et al. Pain, 2009)

Intestinal barrier function in health and gastrointestinal disease

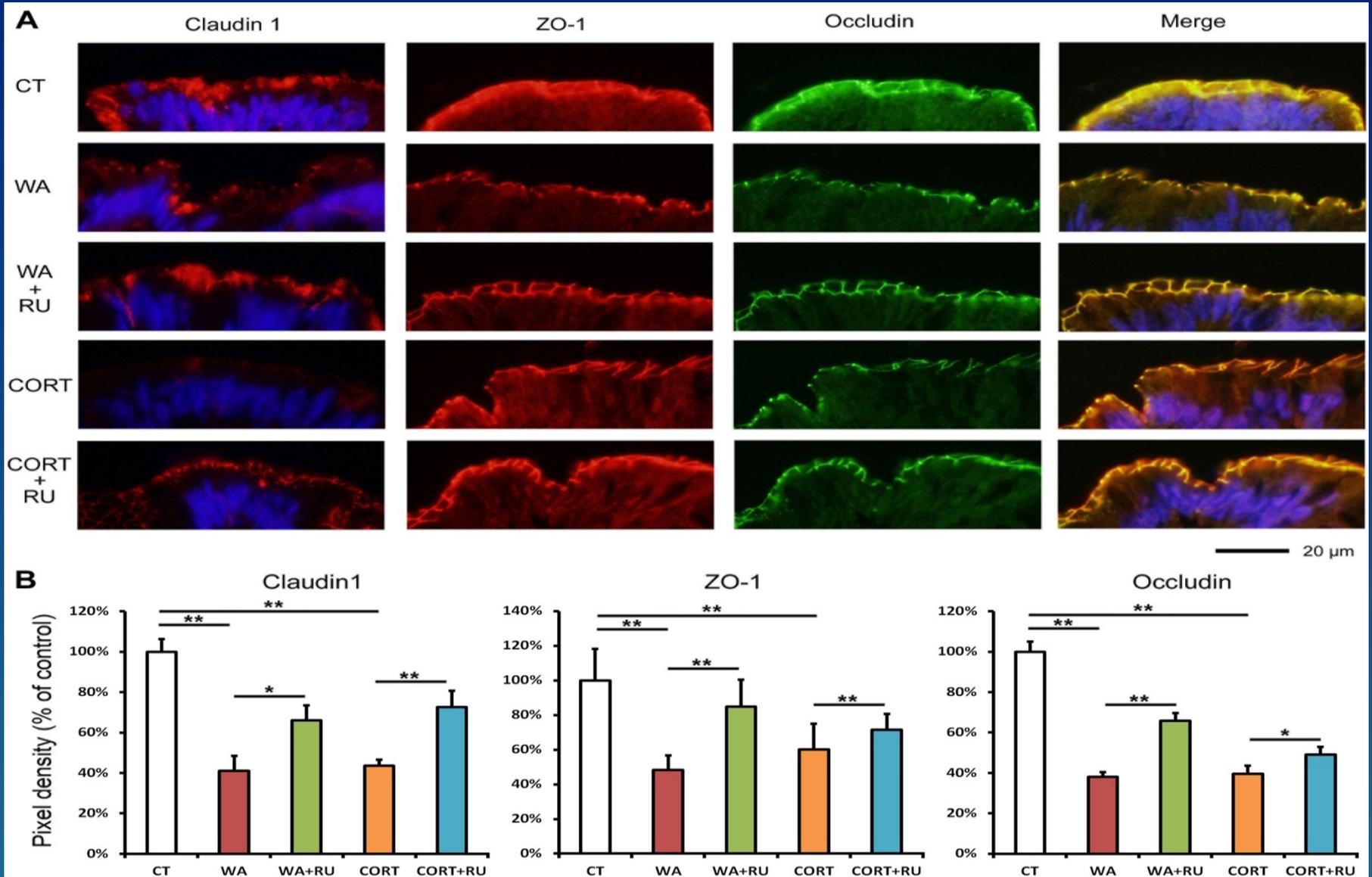


Neurogastroenterology & Motility

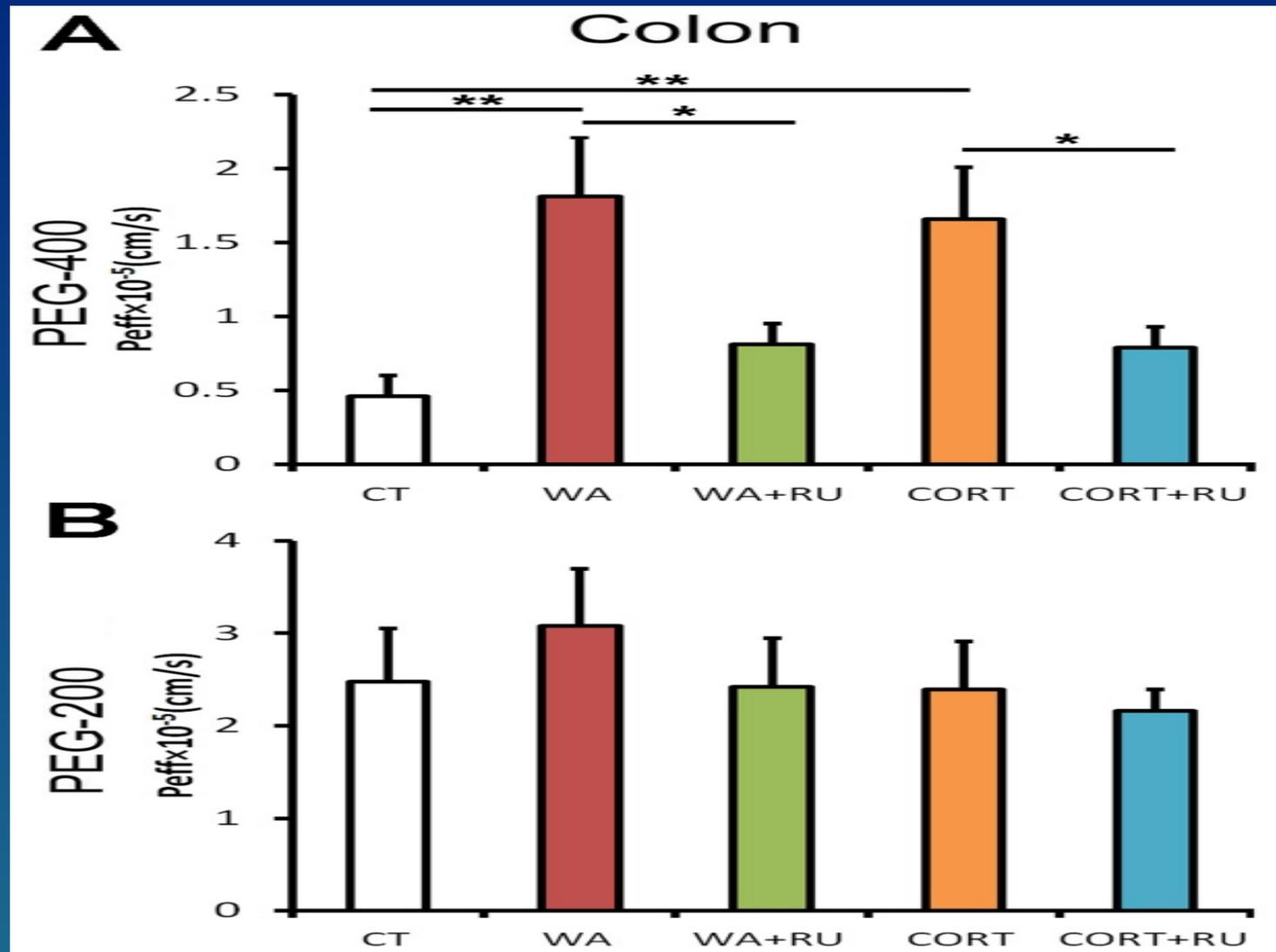
Volume 24, Issue 6, pages 503-512, 14 MAY 2012 DOI: 10.1111/j.1365-2982.2012.01921.x

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2982.2012.01921.x/full#f1>

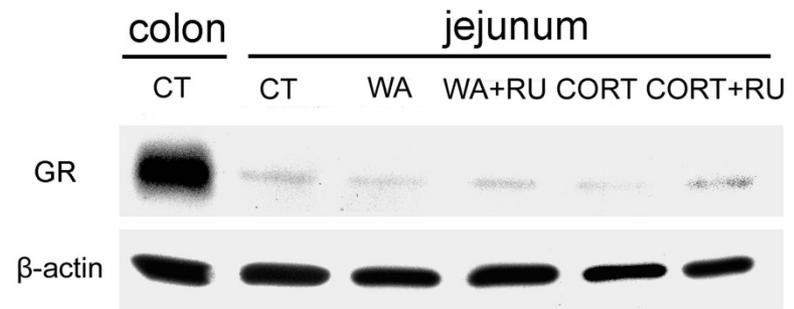
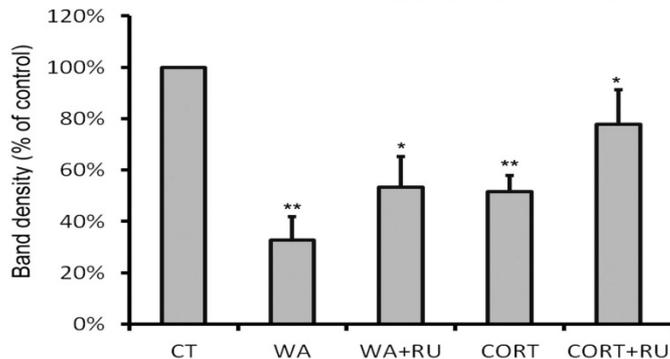
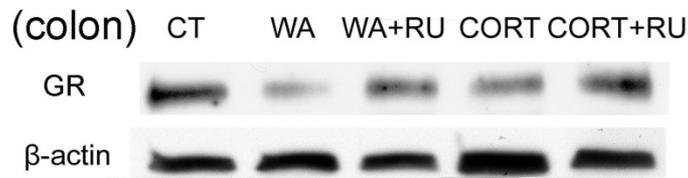
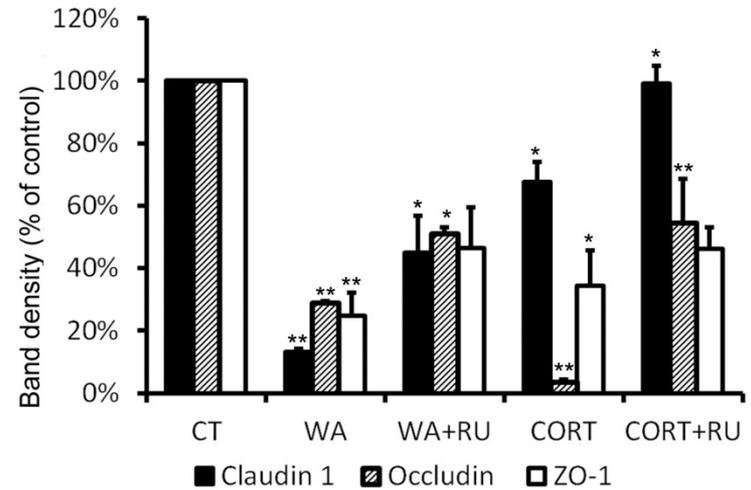
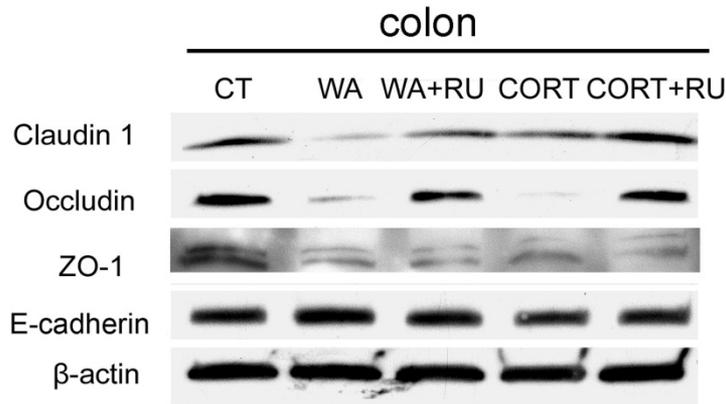
Chronic Stress and Colon Epithelial Tight Junction Profiles (Zheng G et al., NGM, 2013)



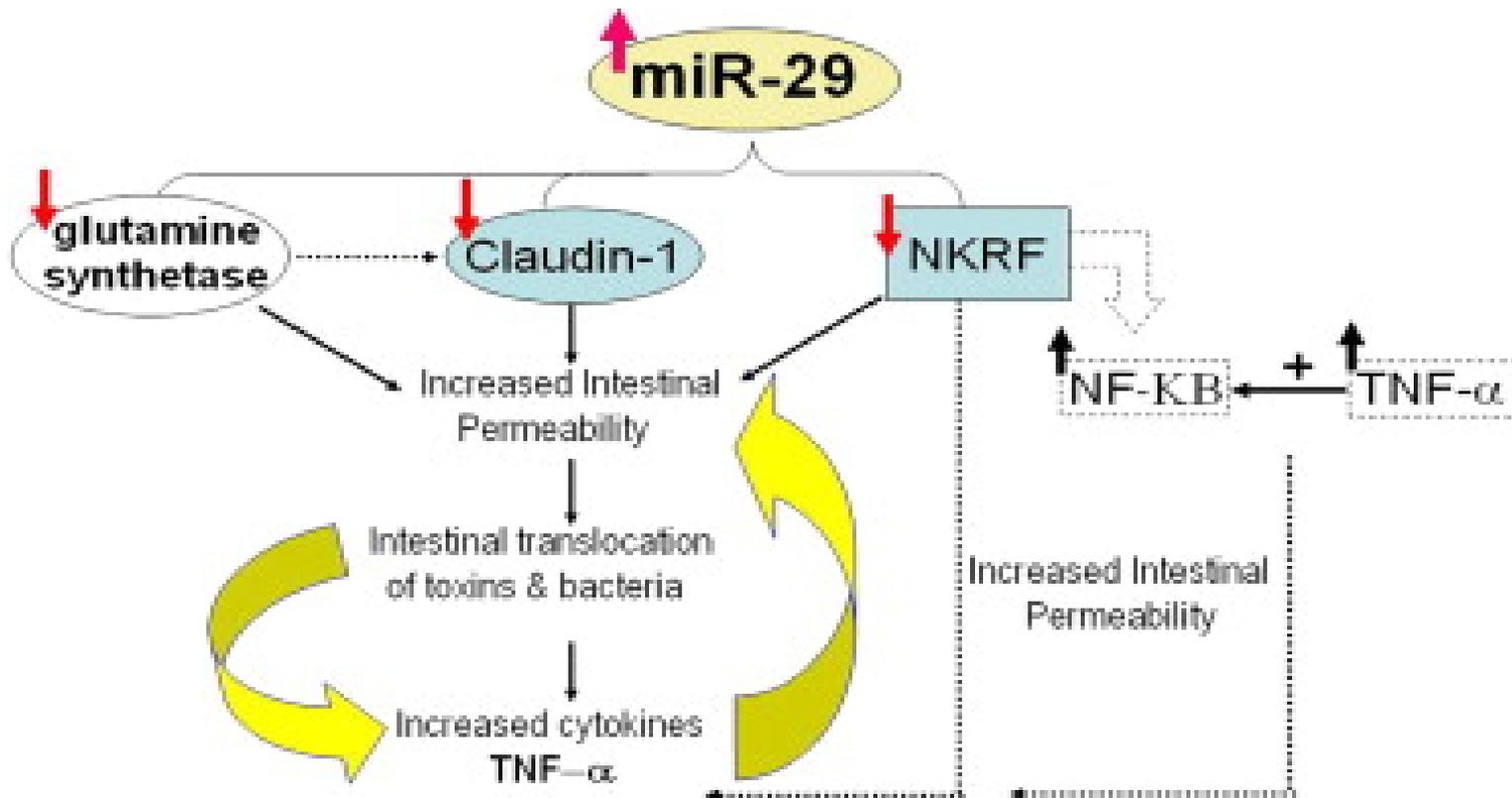
Chronic Stress and Colon Permeability



Chronic Stress, Epithelial Tight Junction Protein and GR Expression



Potential Mechanistic Relationship of miR-29 and Intestinal Permeability



Working model for potential mechanistic relationship(s) between miR-29, NKRF, CLDN1, and glutamine synthetase. The hypothesized relationship between miR29, glutamine synthetase, and CLDN1 is depicted by *dashed arrows/line...*

MicroRNA 29 Targets Nuclear Factor-κB–Repressing Factor and Claudin 1 to Increase Intestinal Permeability

QiQi Zhou , Stefan Costinean , Carlo M. Croce , Alan R. Brasier , Shehzad Merwat , Scott A. Larson , Sarpreet Bas...

Summary

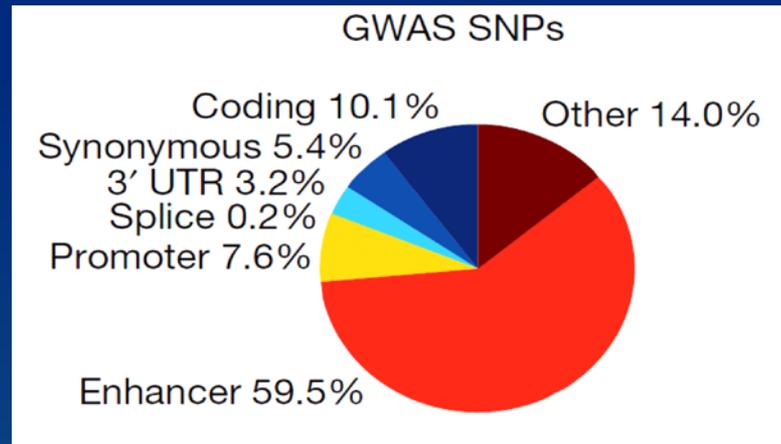
- The chronic intermittent stress rat model demonstrates:
 - Increased methylation of upstream GR and CB1 promoter sites associated with decreased expression of this anti-nociceptive pathway.
 - Enhanced histone acetyltransferase P300-mediated acetylation of H3 in the upstream promoter region of TRPV1 associated with increased expression of this pro-nociceptive pathway and visceral hyperalgesia.
 - These changes are region- and cell-specific and involve nociceptive DRG neurons innervating the colon.
 - Region-specific changes in gut barrier function (colon) : decreased expression in epithelial tight junction proteins and increased paracellular permeability to low molecular weight molecules.

Future Directions

- DNA methylation, DNA-Chromatin compaction and microRNAs operate concurrently in a regional- and cell-specific manner. (Bock C, Nature Genetics, 2012)
- Ethnic, gender and individual differences in epigenetic regulatory pathways. What are the underlying mechanism(s)?
- “Chicken-Egg” Experiments: 1. Are epigenetic-mediated changes in CNS dependent on PNS input or independent?, 2. What’s the role of epigenetic regulation in intestinal barrier dysfunction, i.e. direct and/or indirect?
- What are the mechanism(s) underlying generational and transgenerational epigenetic memory? (Roth ED et al, Neuroepigenetics, 2015, Reizel Y et al. Gene Dev., 2015; Berry S et al. Elife, 2015)

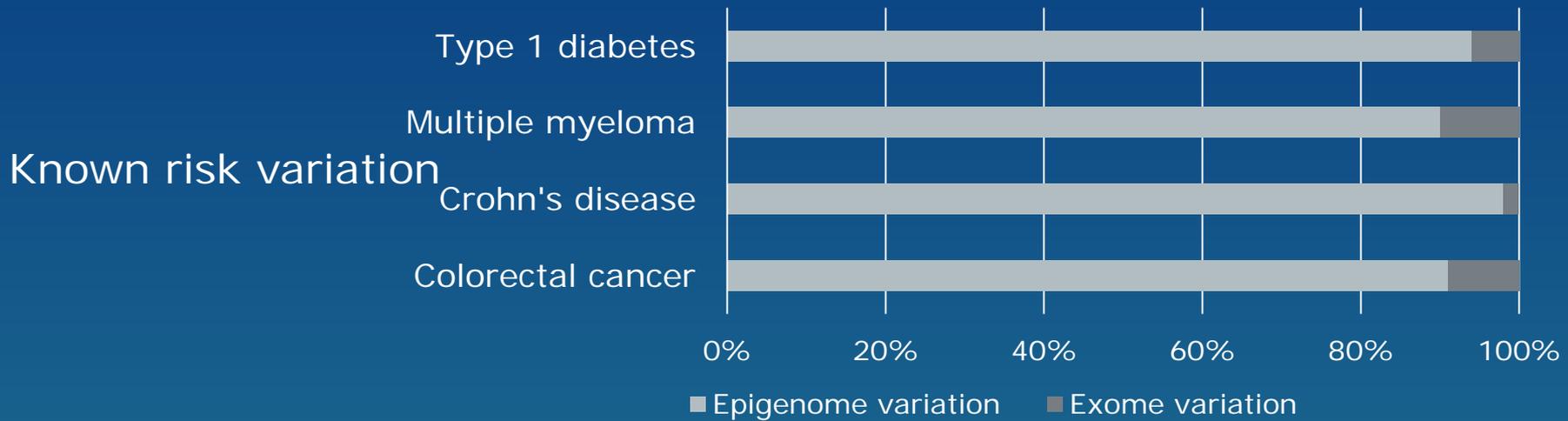
NIH Roadmap Epigenome Project– Summary of Initial Data

1. About 90% of causal SNPs from GWAS are non-coding, and 60% map to cell type-specific enhancers¹:



← Mostly introns and cell-type specific TFBS

2. Inter-individual variation in the epigenome is a more significant predictor of human traits, including disease, than is variation in the exome.^{1,2,3,4}



¹Farh KK H et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature*. 518, 337–343 (2015) doi:10.1038/nature13835

²Yao L et al. Functional annotation of colon cancer risk SNPs. *Nature Comm.* 5, 5114 (2015) doi:10.1038/ncomms6114

³Gascard P et al. Epigenetic and transcriptional determinants of the human breast. *Nature Comm.* 6, 6351 (2015) doi:10.1038/ncomms7351

⁴Seumois G. Epigenomic analysis of primary human T cells reveals enhancers associated with TH2 memory cell differentiation and asthma susceptibility. *Nature Immunol.* 15, 777–788 (2014) doi:10.1038/ni.2937



Acknowledgements

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Effect of Antibiotics on Stress-induced Hyperalgesia

