Epigenetic Changes Associated with Sex and Their Role in Irritable Bowel Syndrome Pathophysiology

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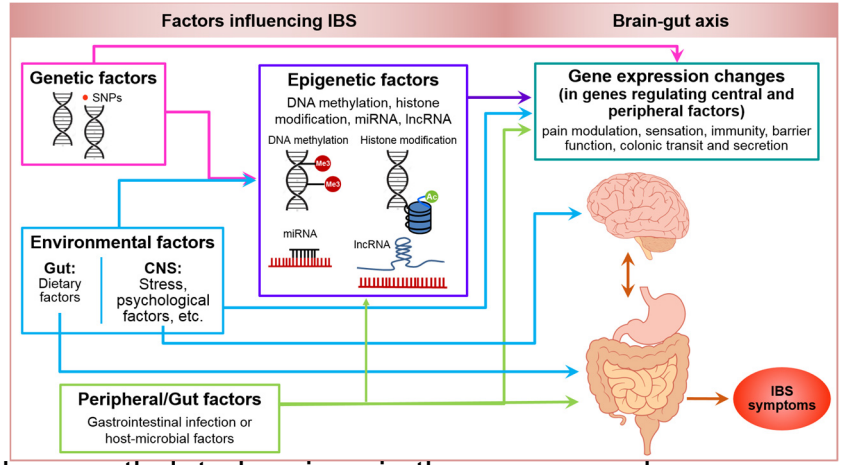
Introduction

- IBS is a female-predominant, stress-sensitive disorder characterized by abdominal pain, altered bowel habits and increased early adverse life events (EALs) [1]
- EALs can lead to long-lasting epigenetic changes in stress-related genes[2]
- Epigenetic modifications, including DNA methylation and histone modification, are inherited changes that can alter gene expression that do not involve changes to the underlying DNA sequence



- Promoters are CpG rich (CpG islands [CGIs]) but relatively unmethylated regions in the genome, whose methylation results in transcriptional repression
- DNA methylation changes associated with sex have been reported[3], however, their role in peripheral pathophysiologic mechanisms in IBS is not clear

References: 1. Park SH, Neurogastroenterol Motil 2016; 2. Liu S, Neurogastroenterol Motil. 2017; 3. Kaz AM, Epigenetics 2014

Aims

- Aims of this study were to identify
  - Differentially methylated genes in the colonic mucosa of IBS patients compared to healthy controls (HCs)
  - Potential DNA methylation-based subtypes within IBS
  - Sex-specific IBS-associated DNA methylation changes
  - Changes in expression of differentially methylated genes associated with IBS and sex

Methods

- 102 patients and 36 HCs underwent sigmoidoscopy with sigmoid colon biopsies
- Two biopsies per patient were used, one for DNA methylation and one for gene expression
- All subjects completed questionnaires and clinical data including IBS symptoms, bowel habits, abdominal pain and bloating, GI symptom severity and psychological symptoms
- DNA methylation from colonic mucosal DNA was measured using HM450 BeadChip (Illumina, Inc.) and gene expression was measured using 3' RNA sequencing
- Associations with clinical traits including IBS symptoms were analyzed using linear regression
- FDR <0.05 or P<0.001 was considered significant, data were analyzed using R software

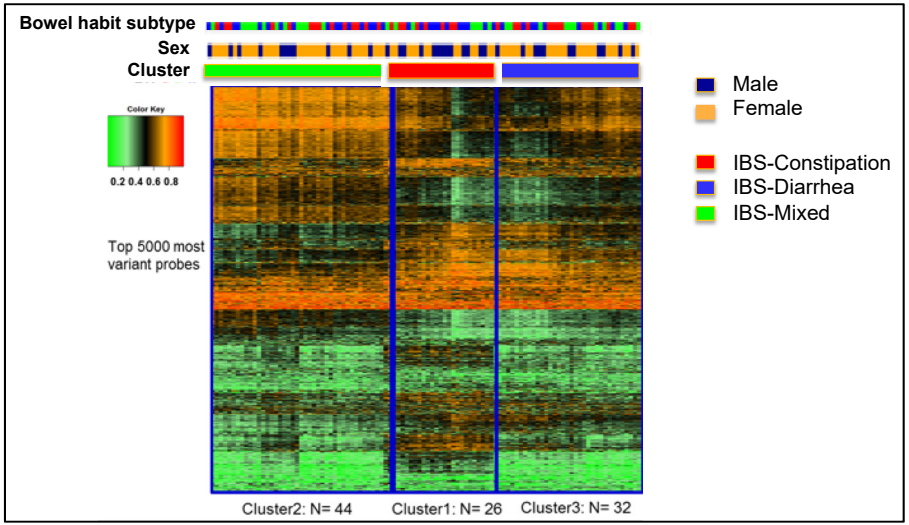
Results

Clinical characteristics Table

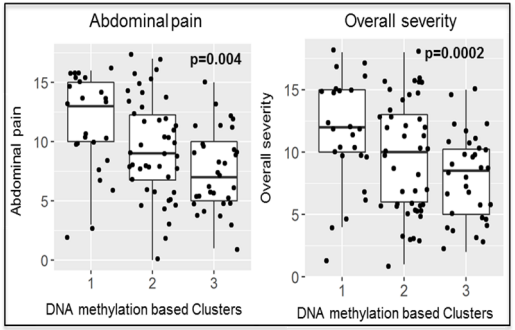
Clinical characteristic	IBS (N=102)	Healthy Controls (N=36)	P value
Age (years) [mean(SD)]	30.88 (10.83)	32.97 (9.38)	0.74
Body mass index (BMI) [mean(SD)]	25.1 (4.77)	26.67 (4.24)	0.14
Female %	65	53	0.11
Bowel Habits % IBS-C IBS-D IBS-M	 33 33 34	-	-
Abdominal Pain (0-20)	9.11 (4.28)	-	-
Overall Severity (0 - 20)	9.54 (4.34)	-	-
Bloating (0-20)	11.06 (4.77)	-	-
ACE Score[1] (0 - 8)	1.91 (1.79)	1.46 (1.77)	0.23

ACE: Adverse childhood events; References: Park SH, Neurogastroenterol Motil 2016

Unsupervised clustering of DNA methylation probes revealed subtypes within IBS



- Cluster 1 was associated with increased abdominal pain scores (scale 0-20, p=0.004) and overall severity (scale 0-20, p=0.0002)
- Cluster 1 was enriched in men (p=0.014)
- Some of the genes hyper-methylated in cluster 1 vs cluster 3 included vasoactive intestinal peptide (VIP), transient receptor potential cation channel, subfamily V, member 4 (TRPV4), which have been associated with IBS pathophysiology

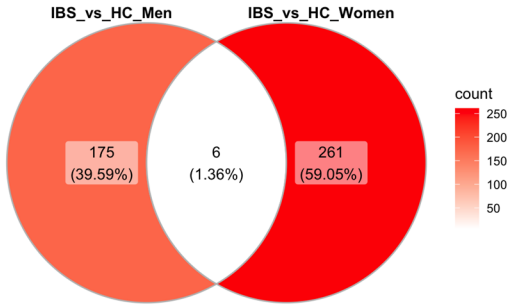


Differentially Methylated CpG positions (DMPs) associated with IBS and sex

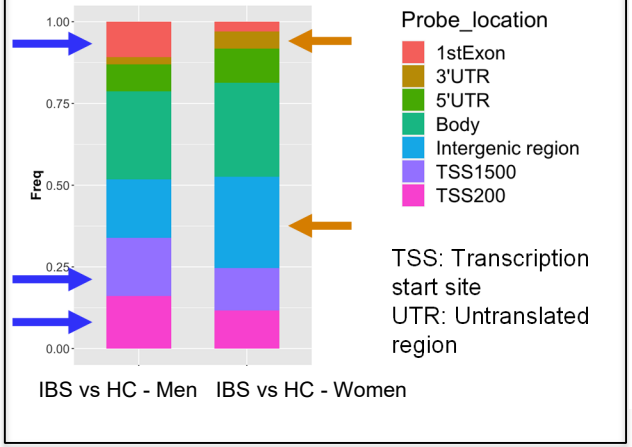
	IBS vs HCs p<0.001	Men vs Women FDR<0.05	IBS vs HC men, p<0.001	IBS vs HC women, p<0.001
Differentially methylated CpG positions (DMPs)	209	3662	230	401

IBS-associated DNA methylation changes are different in men and women

Overlap between IBS-associated differentially methylated CpGs in men and women

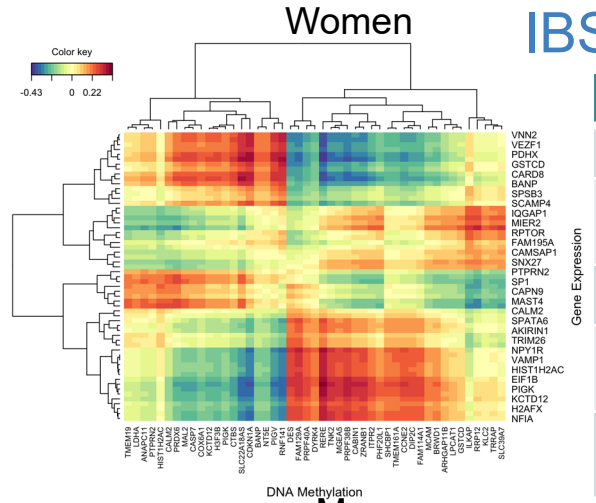


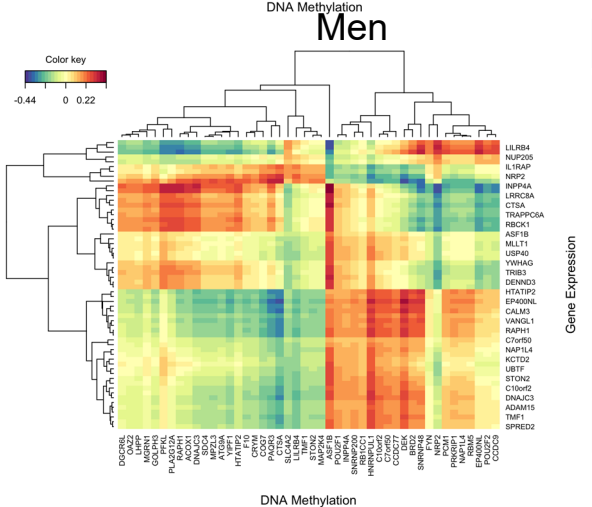
Location of IBS-associated differentially methylated CpGs in men and women



- There was 1% overlap between IBS associated DMPs between men and women
- IBS-associated DMPs in men were located in promoter region (→) , whereas in women, IBS-associated DMPs were intergenic or in 3'UTR (←) [IBS-associated promoter and non-promoter probes in men vs women, p=0.046], suggesting distinct effects of IBS-associated epigenetic changes on gene transcription in men and women

Correlation between DNA methylation and gene expression of IBS markers in women and men





Gene	Name	Function
PHF20L1	PHD finger protein 20-like 1	Regulates DNA methylation
PIGK	phosphatidylinositol glycan anchor biosynthesis class K	Neuronal development
BANP	BTG3 associated nuclear protein	Regulates histone modification
ITPR2	inositol 1,4,5-trisphosphate receptor type 2	Ion channel (Neuro-muscular signaling)
KCTD12	potassium channel tetramerization domain containing 12	Ion channel (associated with stress and rumination[1])

Gene	Name	Function
NRP2	Neuropilin-2	Lymphatic development, inflammatory response
DGKZ	Diacylglycerol Kinase	Kinase activity, immune function
CDKN1B	Cyclin Dependent Kinase Inhibitor 1B	Kinase activity, immune function
DNAJC3	Interleukin-1 receptor accessory protein	Inflammatory response
POU2F2	POU Class 2 Homeobox 2	Immune system

References: 1. Elzari et al., Trans. Psychiatry 2019

Summary and Conclusions

- DNA methylation changes were in the colonic mucosa in IBS patients were associated with sex and symptom severity
- IBS associated DNA methylation changes are distinct in men compared to women
- IBS-related combined DNA methylation and gene expression changes were distinct within men and women
- Immune related pathways were associated with IBS in men, while DNA methylation changes in neuronal pathways were associated with IBS women
- Sex is an important biological variable and plays an important role in DNA methylation mediated mechanisms in IBS pathogenesis
- Further studies are needed to delineate these mechanisms

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