682

FECAL METABOLOMIC ANALYSIS OF SEROTONIN TRANSPORTER DEFICIENT MICE UNDER BASAL CONDITIONS AND CHRONIC COLITIS

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Inflammatory bowel diseases (IBDs) are chronic inflammatory multifactorial diseases caused by genetic, immune, and environmental factors. A decrease in intestinal serotonin transporter (SERT), which controls the extracellular availability of serotonin (5-HT) has been implicated in IBD. We previously showed that SERT deletion in mice altered gut bacterial community structure. The gut microbiota-derived metabolites are functional intermediaries between the microbiota and host. Here, we investigated the impact of SERT deficiency on gut metabolites under basal conditions and chronic colitis mimicking human IBD. Methods: Global metabolic profiles were analyzed by "Metabolon" (Durham, NC) on fecal samples from wild type littermates (WT) and SERT KO mice given water or chronic DSS (2.5% DSS for 5 weeks, n=7-9/group). Data were analyzed by ANOVA contrasts (difference between groups) and by two-way ANOVA ($P<0.05$, $q<0.01$). Results: SERT KO exhibited more severe colitis vs WT as assessed by histological score, myeloperoxidase activity and colon length. There was more pronounced decrease in the mRNA of tight junction proteins (TJs) occludin-1 and ZO-1 in SERT KO DSS vs WT DSS intestine. Metabolic profiling revealed that SERT deficiency alone resulted in extremely low levels of fecal ectoine, a bacterial derived solute that maintains TJ proteins expression. DSS treatment of WT (but not SERT KO) resulted in a significant increase in microbial derived metabolites including phenylalanine, N-acetylphenylalanine, tyrosine derivatives, glutamate, glutamine and benzoate derivatives. An increase in Trimethylamine N-oxide (TMAO), the short chain fatty acids butyrate/isobutyrate, TCA cycle metabolites; and a decrease in several metabolites including spermidine and various primary and secondary bile acids occurred in DSS treated WT and SERT KO to varying degrees; suggesting that these pathways may contribute to the colitis severity in SERT KO mice. Several secondary bile acids, ketone bodies, the metabolites of pterin, riboflavin pathway (FAD and FMN) and fatty acid metabolism pathways were increased in SERT KO (basal and DSS) suggesting genotype related differences in microbial community. We recently showed an impairment of Aryl hydrocarbon receptor (AhR), an IBD susceptible gene, in SERT KO mice, which could be partly due to altered availability of ligands. Indeed, the bacterial derived AhR ligand tryptamine was extremely low in SERT KO (basal and DSS). DSS increased the host derived AhR ligand, kynurenine in WT, but not in SERT KO. Conclusion: These data highlight the impact of serotonergic machinery and SERT inhibition on host physiology and pathophysiology of IBD. The results provide unique insights into gut bacteria derived metabolites and may aid in the development of novel treatment for disorders with altered 5-HT availability (Supported by CCFA and NIH).

683

IDENTIFICATION OF A TRANSMISSIBLE *γδ* INTRAEPITHELIAL LYMPHOCYTE HYPERPROLIFERATIVE PHENOTYPE ASSOCIATED WITH THE INTESTINAL MICROBIOTA

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Background: Intraepithelial lymphocytes expressing the *γδ* T cell receptor (*γδ* IEL) provide a first line of defense to limit intestinal injury and infection. Although the presence of an intact microbiome is not required for *γδ* IEL development, how the microbiota influences this sentinel population is poorly understood. Based on the known role for commensal-induced tonic type I IFN signaling in lamina propria lymphocyte homeostasis, we hypothesized that microbiota-induced IFNα/β receptor (IFNAR) signaling contributes to the maintenance of the *γδ* IEL compartment. **Methods:** Morphometric analysis was performed on the jejunum of GFP *γδ* T cell reporter mice (TcrdEGFP, WT) and TcrdEGFP IFNAR KO mice. 5-Ethynyl-2'-deoxyuridine was administered (0.5 mg i.p.) daily for 5 days to assess *γδ* IEL proliferation *in vivo*. The fecal microbiota of WT and IFNAR KO mice housed in standard and enhanced barrier facilities (SBF or EBF) was analyzed by 16s rRNA sequencing. Vancomycin (400 μg/ml) and meropenem (200 μg/ml) were administered ad libitum in the drinking water. **Results:** Morphometric analysis showed a 2-fold increase in the number of GFP^+ *γδ* IELs in adult IFNAR KO mice compared to WT in SBF. Supporting this, IFNAR KO *γδ* IELs exhibited a 50% increase in proliferation compared to WT. Whereas very few *γδ* IELs populate the SBF WT gut at one week after birth, SBF IFNAR KO *γδ* IELs were increased 11-fold at this early timepoint. Interestingly, IFNAR KO mice rederived into the EBF had a normal *γδ* IEL compartment, indicating that the *γδ* IEL hyperproliferative phenotype occurs independently of IFNAR signaling. The transfer of dirty bedding from SBF IFNAR KO mice to EBF IFNAR KO breeding cages was sufficient to induce the *γδ* IEL hyperproliferative phenotype in both the breeders and their offspring. Moreover, antibiotic treatment prevented the transmission of the *γδ* IEL phenotype to EBF IFNAR KO mice under the same conditions. In separate experiments, separately-housed SBF WT and IFNAR KO mice were crossed to generate F2 littermates. Similar to SBF IFNAR KO mice, *γδ* IEL number was increased 2-fold in adult F2 WT mice. Further, a 71% increase in *γδ* IEL proliferation was observed in F2 WT mice compared to SBF WT. Evidence of both horizontal and vertical transmission of the IEL phenotype led us to analyze the fecal microbiota. Greater microbial diversity was observed in SBF IFNAR KO and F2 littermates compared to SBF WT and EBF mice of either genotype (Shannon Index, $p<0.01$). Moreover, we found 6 amplicon sequence variants (ASVs) that were strongly associated with the *γδ* IEL phenotype ($R^2=0.46$, $p=1.075e-05$). **Conclusions:** We have serendipitously discovered a novel *γδ* IEL hyperproliferative phenotype that arises early in life, and determined that the intestinal microbiota associated with SBF IFNAR KO mice is both necessary and sufficient for the transfer of this *γδ* IEL phenotype.

684

THE USEFULNESS OF COMBINATION BETWEEN FECAL BIOMARKERS AND FECAL IMMUNOCHEMICAL TEST FOR IMPROVING ADVANCED COLORECTAL NEOPLASIA DETECTION IN COLORECTAL CANCER SCREENING: A PROSPECTIVE CROSS-SECTIONAL STUDY

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Background: Fecal immunochemical test (FIT)-based colonoscopy is widely accepted as a primary screening tool for colorectal cancer (CRC) detection. However, FIT could be negative in some patients with advanced colorectal neoplasia (AN). Therefore, we aimed to evaluate the diagnostic performance of combination between fecal biomarkers (fecal transferrin (Tf), fecal calprotectin (Cp) and fecal lactoferrin (Lf)) and FIT to improve advanced colorectal neoplasia (AN) detection. **Methods:** Asymptomatic subjects aged 50-75 years who participated at CRC screening clinic were prospectively enrolled. All subjects were tested with one-time qualitative FIT (cutoff 5 ug/g), Cp (cutoff 50 ug/g), Tf (cutoff 0.4 ug/g) and Lf (cutoff 10 ug/g). The stool collection was obtained within 3 days prior to colonoscopy. All subjects underwent colonoscopy regardless of the stool test result. The endoscopists were blinded to the stool results. We assessed performance of FIT alone and combinations of FIT with Tf (FIT+Tf), Cp (FIT+Cp), and Lf (FIT+Lf) in detecting AN (advanced adenoma and colorectal cancer). AN was defined as adenoma with high-grade dysplasia, villous pattern, size ≥10 mm, or intramucosal cancer. The location of AN was classified as the proximal colon and the distal colon. The region proximal to the splenic flexure and the splenic flexure were defined as the proximal colon. **Results:** A total of 457 subjects (mean age 61.4 ± 6.6, female 71%) were recruited. AN and CRC were found in 43 (9.4%) and 5 (1.1%), respectively. The positivity rates of FIT, Cp, Tf, and Lf were 20.6%, 49.9%, 66.3%, and 10.3% respectively. For overall AN, FIT alone demonstrated sensitivity of 55.8% while FIT+Cp, FIT+Tf and FIT+Lf increased the sensitivity to 81.4% ($p<0.01$), 76.7% ($p<0.01$) and 58.1% ($p=0.48$), respectively (Table 1). For proximal AN, FIT alone demonstrated sensitivity of 64.7% while FIT+Cp, FIT+Tf and FIT+Lf increased the sensitivity to 82.4% ($p<0.01$), 82.4% ($p<0.01$), and 64.7% ($p=1.00$), respectively. The negative predictive value (NPV) for overall AN in FIT alone, FIT+Cp, FIT+Tf and FIT+Lf was 94.8%, 96.0%, 93.2%, and 94.7%, respectively. The number of needed colonoscopies to detect one AN increased from 3.9, by using FIT alone, to 7.3 by using FIT+Cp indicating that using FIT alone and FIT+Cp could reduce 63% and 31% unnecessary colonoscopy when compared with primary colonoscopy, respectively. For CRC, sensitivity of FIT, FIT+Cp, FIT+Tf, and FIT+Lf was 80%, 80%, 100%, and 80% respectively. **Conclusion:** The combination of FIT+Cp is the most sensitive test to detect AN among all three

Advanced colorectal neoplasia detection	Individual fecal biomarker test				Combination of fecal biomarker test			
	FIT	Cp	Tf	Lf	FIT + Cp	FIT + Tf	FIT + Lf	FIT + Cp + Tf + Lf
Positivity rate (%)	20.6	49.9	66.3	10.3	56.0	67.8	25.8	75.5
Accuracy (%)	80.5 (76.6-84.1)	53.0 (48.3-57.6)	38.3 (33.8-42.9)	83.4 (79.6-86.7)	49.9 (45.2-54.6)	37.2 (32.8-41.8)	75.7 (71.5-79.6)	31.1 (26.9-35.5)
Sensitivity (%)	55.8 (39.8-70.9)	65.1 (49.1-79.0)	74.4 (58.8-86.5)	16.3 (6.8-30.7)	81.4 (66.6-91.6)	76.7 (61.4-88.2)	58.1 (42.1-73.0)	86.1 (72.1-94.7)
Specificity (%)	83.1 (79.1-86.6)	51.7 (46.8-56.6)	34.5 (30.0-39.3)	90.3 (87.1-93.0)	46.6 (41.7-51.6)	33.1 (28.6-37.9)	77.5 (73.2-81.5)	25.4 (21.2-29.8)
NPV	94.8 (92.8-96.2)	93.5 (90.4-95.6)	92.9 (88.5-95.7)	91.2 (90.1-92.3)	96.0 (92.8-97.9)	93.2 (88.7-96.0)	94.7 (92.6-96.2)	94.6 (89.1-97.4)
PPV	25.5 (19.6-32.5)	12.3 (9.9-15.1)	10.6 (8.9-12.5)	14.9 (7.7-26.8)	13.7 (11.8-15.8)	10.7 (6.9-12.5)	21.2 (16.5-26.8)	10.7 (9.5-12.0)
False negative rate (%)	44.2 (29.1-60.1)	34.9 (21.0-50.9)	25.6 (13.5-41.2)	83.7 (69.3-93.2)	18.6 (8.4-33.4)	23.3 (11.8-38.6)	41.9 (27.0-57.9)	13.9 (5.3-27.9)
Cancer missed rate (%)	20.0	20.0	0.0	40.0	20.0	0.0	20.0	0.0
Number needed to screen	19.0	16.3	14.3	65.3	11.8	12.5	16.6	12.4
Number needed to colonoscope	3.9	8.1	9.5	6.7	7.3	9.4	4.7	9.4

Table 1: Diagnostic performance of fecal immunochemical test, fecal calprotectin, fecal transferrin and lactoferrin for overall advanced colorectal neoplasia

685

A SENSITIVE AND QUANTITATIVE NON-INVASIVE MULTIMODAL BLOOD TEST THAT DETECTS CANCER AND RECAPITULATES ADENOMA PROGNOSIS AVAILABLE FROM COLONOSCOPY

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Background A new understanding of the natural history of colorectal cancer based on i) initial expansion and selection of genetically diverse intermixed sub-clones, ii) early epithelial cell dissemination with late metastasis, and iii) the integrated role of intrinsic adenoma and extrinsic microenvironment factors emphasizes the continual emergence of malignant features rather than dichotomous pre-malignant and malignant states. Our aim was to evaluate a blood-based strategy in equipoise with colonoscopy that provides prognostic information for progression to colorectal cancer (CRC) analogous to adenoma size and number for patient management. **Methods** A single-center, IRB-approved, prospective, blinded study was conducted at the Veterans Affairs Palo Alto Health Care System. Interim results for 458 patients with no prior diagnosis of CRC who were scheduled for outpatient colonoscopy are presented. Indications for colonoscopy were 86% asymptomatic and 14% with symptoms or positive-FIT. Patients had blood drawn immediately prior to colonoscopy. The test analyzes two biomarkers: circulating gastrointestinal epithelial cells (CEC), validated somatic mutations of cell-free DNA and uses history of advanced adenomas and incident risk (age, gender) for CRC to calculate a *FirstSight* Score. Multivariate ordinal and nominal logistic regression methods were used to assess the degree of association between the pre-defined *FirstSight* Scores and adenoma size and number, adjusting for DNA mutation status.

Results The sensitivity for CRC (TNM stage I-IV) was 100% (14/14, 78.5%-100%) and for advanced adenomas was 74.2% (49/66, 62.3%-83.3%). The specificity (negative colonoscopy or nonneoplastic findings) was 90.8% (139/153, 85.2%-94.5%). Linear and proportional odds regression models were used to model adenoma size, number and pathology classification versus *FirstSight* Scores. There is a significant association between *FirstSight* Scores and adenoma size (LR x^2= 61.5, p-value <0.00001), number of adenomas (LR x^2 = 38.1, p-value < 0.00001), and ordinally increasing pathology classification (LR x^2 = 101.3, p-value < 0.00001) (Figure 1, 2). These results suggest that *FirstSight* Scores are providing predictive information of adenoma size, number, and cancer prognosis.

Conclusions A novel noninvasive multimodal blood-based assay that analyzes CEC signals and cell-free DNA for somatic mutations and integrates history of advanced adenomas and SEER CRC incidence risk is significantly correlated with adenoma size and number. CEC number may reflect promotion of cell dissemination by extrinsic factors of the adenoma microenvironment. The opportunity to detect CRC with high sensitivity and specificity as well as to discern adenoma to cancer prognostic information for the continuum of early disease may more precisely inform timely colonoscopy and subsequent interval testing.

Study Results, N=458 Disease Category	# of Subjects	Index Adenoma Size, mean (mm)	Number of Adenomas, mean	*FirstSight* Score, mean
Negative Colonoscopy	101	-	0	62.7
Non-Neoplastic Findings (including hyperplastic polyp <1cm)	52	3.5	1.8	61.9
Non-advanced Adenomas (adenomas <1cm & <25% villous features & low-grade dysplasia)	225	4.8	3.1	70.9
Advanced Adenomas (≥ 1cm adenomas or high-grade dysplasia or >=25% villous features)	66	17.5	4.7	77.2
Colorectal Cancer (TNM stage I-IV)	14	34.5	3.7	85.0

Figure 1. Study Results for CellMax VA N458

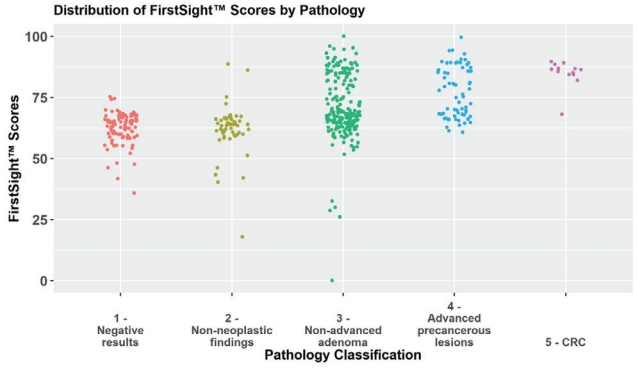

Figure 2. Distribution of FirstSight Scores by Pathology

686

INCREASED POLYP DETECTION IN A WESTERN POPULATION USING A REAL-TIME ARTIFICIAL INTELLIGENCE-BASED SYSTEM DURING COLONOSCOPY: A PILOT STUDY

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Background and Aims: Artificial intelligence (AI) is a rapidly growing field with medical applications in gastrointestinal endoscopy. Computer-aided detection (CAD) has the potential to improve polyp detection rates and decrease miss rates in colonoscopy. Only a few studies have reported real-time CAD during colonoscopy in a clinical setting. We investigated the efficacy of a novel real-time CAD system during colonoscopy in a Western population.

Methods: This was a single-arm prospective study of patients who underwent colonoscopy with a real-time CAD system. The AI-based system was previously trained to detect neoplastic polyps (adenomas and serrated polyps) using colonoscopy videos that were collected and labeled manually. The CAD system output was displayed in real time during colonoscopies on a second monitor placed immediately alongside the primary monitor. For this pilot study, we enrolled 300 patients at two centers to undergo elective colonoscopy with the CAD system. The results were compared to 300 historical controls consisting of consecutive procedures performed by each of the participating endoscopists at the same center within 12 months prior to onset of study. The primary outcome was the mean number of adenomas per colonoscopy.

Results: Use of real-time CAD was associated with a trend towards increased adenoma detection (1.35 vs 1.07 polyps per colonoscopy, p = 0.10). There was a significant increase in detection of serrated polyps (0.15 vs 0.07, p = 0.02), and in detection of all neoplastic (adenomatous and serrated) polyps (1.50 vs 1.14, p = 0.04), compared to the control group. More non-neoplastic polyps were also detected with CAD (1.08 vs 0.57, p < 0.0001). There was no difference in the number of polyps detected that were 10 mm or larger in size between the groups.

Conclusions: A real-time CAD system can increase detection of neoplastic polyps during colonoscopy in a Western population. This increase is largely attributed to detection of polyps smaller than 10 mm. Significantly more serrated polyps were also detected using the CAD system compared to historical controls. Based on the results of this pilot study, we recommend proceeding with a larger randomized controlled trial to definitively evaluate the potential benefits of CAD.

Figure 1. Representative examples of polyps detected using CAD system.
A. Proximal colon adenoma ≤ 6 mm. B. Flat (Paris IIa) distal colon adenoma ≥ 10 mm. C. Flat (Paris IIa) proximal colon serrated polyp 6 – 9 mm. D. Flat (Paris IIa) proximal colon serrated polyp 6 - 9mm with overexposure artifact due to surface reflection.

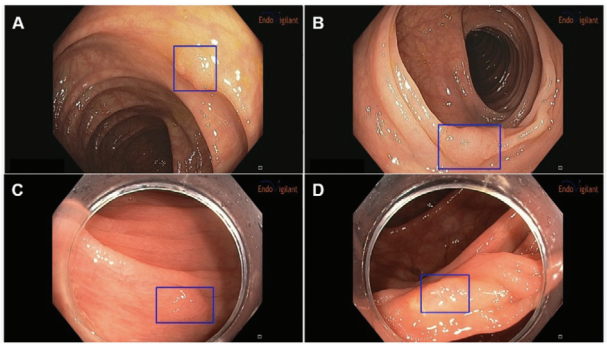

Table 1. Polyps found during colonoscopy (values given as mean + SD number of polyps found per colonoscopy)

	Study Group	Control Group	Significance
Adenomas	1.35 ± 2.2	1.07 ± 1.8	NS (p = 0.099)
Adenomas and serrated polyps	1.50 ± 2.3	1.14 ± 1.9	p = 0.038
Serrated polyps	0.15 ± 0.52	0.070 ± 0.31	p = 0.023
Non-adenomatous non-serrated polyps	1.08 ± 1.72	0.57 ± 1.07	p < 0.0001
Adenomas and serrated polyps ≥ 10 mm	0.20 ± 0.73	0.19 ± 0.74	NS (p = 0.91)
Adenomas and serrated polyps 6 - 9 mm	0.32 ± 0.92	0.23 ± 0.59	NS (p − 0.14)
Adenomas and serrated polyps < 5 mm	0.98 ± 1.53	0.71 ± 1.27	p − 0.021
Adenomas and serrated polyps in proximal colon	0.98 ± 1.55	0.80 ± 1.41	NS (p = 0.13)
Adenomas and serrated polyps in distal colon	0.52 ± 1.15	0.34 ± 0.81	p = 0.027
Polypoid (Paris I) polyps ≥ 6 mm	0.44 ± 1.2	0.35 ± 0.8	NS (p − 0.31)
Flat (Paris IIa/b/c) polyps ≥ 6 mm	0.09 ± 0.3	0.09 ± 0.8	NS (p = 0.95)

687

NARROW BAND OR BLUE LIGHT IMAGING FOR DETECTION OF PROXIMAL COLONIC LESION: A PROSPECTIVE RANDOMIZED TANDEM COLONOSCOPY STUDY

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Background: Blue light imaging (BLI) is a new image-enhanced endoscopy which enable better visualization of vascular and mucosal patterns, focusing on the short wavelength absorption of hemoglobin at 410nm. It is similar to narrow band imaging (NBI) which uses 415 and 540nm narrow band illumination. However, there is no direct comparison between BLI and NBI. We compare the proximal colonic polyp (PDR) and adenoma (ADR) detection rates of BLI and NBI, with reference to conventional white light imaging (WLI). **Methods**: It is a planned interim analysis of an ongoing industry-independent, single-center, prospective randomized study of tandem examination of the proximal colon. Eligible patients were randomized in 1:1:1 ratio to receive NBI, BLI or WLI during first withdrawal of the proximal colon, defined as cecum to splenic flexure. Second examination of the proximal colon and the examination of remaining distal colon were performed under WLI in all patients. We included all patients, aged >40, who were scheduled for colonoscopy for all indications including screening colonoscopy. Patients with prior colectomy or familial colorectal cancer were excluded. Primary outcomes were PDR and ADR between BLI and NBI, as compared to WLI. **Results**: 673 patients who had tandem examination of the proximal colon were included in this interim analysis (BLI: 225; NBI: 224; and WLI: 224). The mean age of patients was 64.9 years with 53% male, and 29.4% underwent colonoscopy for screening. WLI has the lowest PDR (39.7%) and ADR (31.2%) among three groups. The corresponding