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CHARACTERISTICS ASSOCIATED WITH COMPREHENSIVE STOOL ANALYSIS FINDINGS IN ADULT INTEGRATIVE MEDICINE PATIENTS

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Kristin L. Young

Submitted to the graduate degree program in the Clinical Research and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science

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Chairperson K. Allen Greiner, MD, MPH

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Sue-Min Lai, PhD, MS, MBA

_____

Jeanne Drisko, MD

Date Defended: December 1, 2011

The Thesis Committee for Kristin L. Young, PhD,
Certifies that this is the approved version of the following thesis:

CHARACTERISTICS ASSOCIATED WITH COMPREHENSIVE STOOL ANALYSIS FINDINGS IN ADULT INTEGRATIVE MEDICINE PATIENTS

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Chairperson K. Allen Greiner, MD, MPH

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ABSTRACT

This pilot study explores relationships between clinical and socio-demographic characteristics and abnormal comprehensive stool analysis (CSA) results of patients at the Integrative Medicine Clinic at the University of Kansas Medical Center, to evaluate comprehensive stool analysis as a potential tool to stratify patients by risk of developing gastrointestinal disease as a first step to defining personalized risk reduction strategies. The primary hypothesis was that Integrative Medicine Clinic patients with lower socioeconomic status, chronic disease, poor nutritional status, and/or general health risks would be more likely than other patients to have abnormal bacterial counts and abnormally low levels of short-chain fatty acids. Data were abstracted from paper charts in the Integrative Medicine Clinic, representing current adult patients in the clinic with comprehensive stool analysis reports in their files (N=295). Analysis of the available data revealed that not all relevant data (race/ethnicity, occupation, household income) were recorded for all patients, making correlations between the outcomes of interest and certain socio-demographic variables impossible. However, multivariable relative risk regression revealed that stomach pain, fecal pH, and methylation insufficiency were significant predictors of abnormally low levels of total short chain fatty acids, while abnormal lactoferrin levels, and detoxification genome markers NAT2*K268R and NAT2*I114T were significant predictors of abnormally low levels of the beneficial *Bifidobacter* bacterial species. Future work should establish CSA baseline results in a larger, more generalizable population and follow a cohort prospectively to establish the relationship between abnormal CSA and disease risk.

This thesis is dedicated to my husband, Brandon, for his unwavering love and support, and to Kaity, Brae, Hannah, and Charlotte, for making me proud to be able to tell my children that their mom is a scientist.

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INTRODUCTION

According to the Surveillance, Epidemiology, and End Results (SEER) Program and the National Center for Health Statistics (http://seer.cancer.gov), colorectal cancer (CRC) is the third most common cancer diagnosed regardless of gender, and is the third leading cause of cancer-related death. It is estimated that approximately $8.4 billion is spent yearly on colorectal cancer treatment in the United States alone (http://progressreport.cancer.gov). Preventive measures involving education, routine colonoscopy, fecal occult blood testing, and behavioral changes to improve colon health have become imperative in reducing the incidence and treatment costs of CRC. As a result, comprehensive stool analysis (CSA) has become a useful tool in evaluating the general health and integrity of the digestive tract, examining both structural and functional parameters which may further play a role in prevention of gastrointestinal symptoms and CRC (Rafter, 2002; Rowland, 2009).

Increased research interest has recently been focused on the impact of gastrointestinal bacteria on health (Brady et al., 2000; Pregliasco et al., 2008). The human microbiota comprises approximately 10^{14} bacterial cells, with gastrointestinal tract colonization representing around 75% of the total microbes (Naito et al., 2010). The majority of gastrointestinal bacteria fall into 6 major species: *Bacteriodes, Bifidobacterium, Eubacterium, Clostridium, Lactobacillus, and Fusobacterium* (Wallace et al., 2011). The bacterial numbers steadily increase from duodenum (10^2 bacteria/gram) to colon (~10^{12} bacteria/gram). *Bacilli* are enriched in the small intestine while *Bacteriodes* are enriched in the colon (Frank and Pace, 2001). The mucus layer generates additional heterogeneity by separating the bacteria confined to the intestinal lumen from those able to invade below the mucus and attach to the epithelium (Swidsinski et al., 2005). Microbiotic digestion of dietary fiber and modulation of dietary lipids also contribute to bacterial

diversity (Vella and Farrugia, 1998; Bäckhed et al., 2004; Turnbaugh et al., 2006; McFarland, 2008).

The gut is populated by bacteria shortly after birth, and the bacterial composition of the gut flora is established by around two years of age (Mackie et al., 1999; Fanaro et al., 2003). The microbiota composition of each individual is relatively constant over time and is influenced by both individual characteristics and by exposures early in life (Eckburg et al., 2005; Ley et al., 2006; Kelsall, 2008). Studies now suggest that health promoting beneficial digestive tract microflora can influence various GI processes and may also relate to the production of adequate amounts of short-chain fatty acids, and thus promote nutrient absorption through production of digestive enzymes, vitamin synthesis, and immune function, while also inhibiting colonization by pathogens (Weaver et al., 1988; Delzenne et al., 2003; Boutron-Ruault et al., 2005; Lim et al., 2005; Hamer et al., 2008; Liong, 2008; Corfe et al., 2009; Worthley et al., 2009; Garrett et al., 2010).

Few human trials have been conducted to further explore the role of the short-chain fatty acids (SCFA) themselves, or the profile of beneficial bacteria required to produce them, in helping to prevent colorectal cancer or other gastrointestinal disorders (Delzenne et al., 2003; Geier et al., 2006; Rafter et al., 2007; Fotiadis et al., 2008). Research has demonstrated, however, that one of the short-chain fatty acids (butyrate) is a preferred energy source of colonocytes (Greer and O'Keefe, 2011). Other studies have demonstrated that certain bacterial species have been linked to increased risk of gastrointestinal diseases, including colorectal cancer, through production of toxic metabolites or promotion of chronic inflammation that patients with CRC have modified bacterial profiles (Rowland, 2009; Castellarin et al., 2011; Kostic et al., 2011; Marchesi et al., 2011; Sobhani et al., 2011; Wang et al., 2011). *Bifidobacterium* and

Lactobacillus species have been shown to produce low levels of carcinogen-forming metabolites and to bind to certain carcinogens and physically prevent their absorption (Liong, 2008), and have consequently been of particular interest for their potential health benefits. Therefore, bacteria and the metabolites these organisms produce in the gut may be an important modifiable factor related to CRC risk. In addition, while bacterial profiles appear stable, relative distribution of bacterial types can be influenced by various environmental exposures including diet, medication, and smoking behavior (Parry et al., 2005; O'Keefe, 2008; Jernberg et al., 2010). Many of these biological and environmental factors, if characterized and understood through methods including comprehensive stool analysis, could potentially be clinically modified or considered in personalized screening approaches and risk reduction strategies.

As a first step in the development of potential personalized risk reduction strategies for general GI health, the aim of the present study was to examine relationships between patient clinical and socio-demographic characteristics and CSA findings in a group of patients evaluated in the Integrative Medicine Clinic at the University of Kansas Medical Center, with the hypothesis that Integrative Medicine Clinic patients with lower socioeconomic status, chronic disease, poor nutritional status, and/or general health risks would be more likely than other patients to have abnormal bacterial counts and abnormally low levels of short-chain fatty acids.

METHODS

This exploratory pilot study included a convenience sample of adult (age 18 or older at the time of first clinic visit) Integrative Medicine patients with charts currently on file in the Integrative Medicine Clinic at the University of Kansas Medical Center (KUMC). All charts were reviewed for the presence of a comprehensive stool analysis report, and those lacking such a report were excluded. A total of 295 patient charts were included in this retrospective cross-

sectional study. Data from paper based medical charts were abstracted for each patient by a research assistant, who recorded demographic and general health information from each patient's initial clinic intake form, clinical data from each patient's initial CSA laboratory report, personal and family history of GI symptoms/disease, physiological profile, and genetic detoxification profile. Demographic data included patients' age, gender, race/ethnicity, marital status, and level of education. General health information abstracted from patient charts included height and weight, smoking status and alcohol use, symptoms of headache, allergies, sinus issues, low back pain, and arthritis. The physiological profile consisted of a food allergy panel (KU PIM IgG Food Panel II, IBT Laboratories, Lenexa, KS), a neurotransmitter metabolite panel (epinephrine, norepinephrine, and histamine) (NeuroScreen Expanded, NeuroScience, Inc., Osceola, WI), plus additional general chemistry (copper, zinc), cardiac (homocysteine), and vitamin and mineral panel (vitamins A, B complex, D, E) markers. The genetic detoxification profile (Genovations Detoxigenomic Profile, Genova Diagnostics, Asheville, NC) characterizes 22 single nucleotide polymorphisms (SNPs) associated with metabolism of toxic compounds, including enzymes involved in hydroxylation (cytochrome P450 enzyme family), methylation (catechol-O-methyl transferase) and acetylation (N-acetyl transferase). Data from the abstraction sheets were subsequently entered into a Microsoft Excel spreadsheet for data cleaning, and then converted to a SPSS data file for analysis. This study was approved under full committee review by the Institutional Review Board of the University of Kansas Medical Center.

CSA reports were received from two different laboratories (Doctor's Data and Metametrix), depending on when the patient was seen at the Integrative Medicine clinic. While both labs reported the same basic information, some of the variables did not have the same units. Specifically, the variables with differing values include: Elastase 1 - (µg/ml – Doctor's Data,

µg/g – Metametrix), N-Butyrate – (mg/ml - Doctor's Data, mM/g - Metametrix), *Lactobacillus* and *Bifidobacter* – (0-4+ scale – Doctor's Data, millions of colony forming units/gram of stool – Metametrix), and total short chain fatty acids (TSFCA) – (mg/ml – Doctor's Data, mM/g – Metametrix). Elastase 1 and N-Butyrate values were in units that were not able to be converted between the two labs, and so were excluded from additional analyses. *Lactobacillus* and *Bifidobacter* counts were of particular interest as outcome variables, so the Metametrix data were converted to a 0-4 scale based on quintile values obtained from the laboratory. In addition, a new binary outcome variable indicating clinically low values (<1), were created for both *Lactobacillus* and *Bifidobacter* to reflect abnormal levels of each of these beneficial bacterial species. For TSCFA, the third outcome variable, abnormal values were determined based on the reference ranges for each lab, and a new binary variable was created to indicate those patients with abnormally low TSCFA levels.

Outcome variables (abnormal TSCFA, abnormal *Lactobacillus* and abnormal *Bifidobacter*) were coded as binary conditions, where abnormal = 1 and normal = 0, and because the frequency of each of these outcomes was found to exceed 10%, relationships between predictor and outcome variables were evaluated using relative risk regression with an underlying log binomial distribution. Bivariable relative risk regression analysis between each predictor variable and the outcome variables of interest were performed to identify predictors with $p <$ 0.20 for inclusion in multiple logistic regression analysis. Multiple relative risk regression models were constructed to explore predictive factors on abnormal CSA results. Relative risk regression models were evaluated using the Bayesian Information Criterion (BIC) statistic, which provides an asymptotic approximation to the Bayesian posterior probability of a particular

candidate model (Schwarz, 1978). A regression model with the lowest BIC is considered the best fit for the data. Statistical analysis was conducted in SPSS v. 18 and SAS v. 9.2.

RESULTS

Frequencies of categorical predictor variables and mean and standard error of continuous predictor variables used in regression modeling are presented in Table 1. Predictor variables which had more than 100 missing values or fewer than 25 individuals in a particular category were excluded from the primary analysis (indicated by * in Table 1). Because only a subset of the sample (N=95) had data from the detoxification genome profile, these data were analyzed separately as a subgroup analysis. Genetic detoxification profile markers, which had fewer than 10 in a particular category were excluded from this analysis (indicated by * in Table 1). Using the Kolmogorov-Smirnov normality test, all of the continuous predictor variables were found to not be normally distributed, with the exception of age and pH. Because of this, and because there are clinically significant cut-off values for abnormal levels of the physiological and CSA continuous variables, these were converted to binary normal/abnormal variables for further analysis. In addition, pilot research in the Integrative Medicine Clinic has indicated that abnormal levels of histamine, homocysteine, and norepinephrine/epinephrine ratio are associated with methylation insufficiency, so a composite variable (methylation status) was created to indicate when 2 of these 3 variables were in the abnormal range. The biochemical process of methylation is important for production of phospholipids, epigenetic control of gene expression, and synthesis of proteins and various neurotransmitters, and so may have an effect on general health status (Bottiglieri, 2002).

Table 1. Predictor variables used in regression modeling.

Demographic	*N*	*Total N*	*Gastrointestinal Symptoms*	*N (Yes)*	*N (No)*
Gender	Male =91	295	Constipation	131	163
Race/ Ethnicity*	White =84	89	Diarrhea	101	193
Marital Status	Married = 210	290	Heartburn	64	230
Education	College Graduate =126	286	Stomach Pain	107	186
	Mean (SE)	*Total N*	Nausea	53	240
Age	50.51 (0.867)	295	Vomiting	27	266
			Bloating	115	179
General Health	*Mean (SE)*	*Total N*	*Comprehensive Stool Analysis*	*N (Yes)*	*N (No)*
Height*	66.91 (0.331)	160	Yeast	129	166
Weight	158.68 (2.378)	275	Parasites	39	255
General Health	*N (Yes)*	*N (No)*	Dysbiotic Flora	84	207
Smoker*	13	280	Fecal Sig A	215	77
Alcohol	221	72	WBC*	2	291
Arthritis	87	207	Mucus*	3	290
Low Back Pain	139	155	Occult Blood*	11	283
Allergic Rhinitis*	18	276	*Comprehensive Stool Analysis*	*Mean (SE)*	*N*
Sinus Issues	105	189	pH	6.52 (0.327)	294
Headache	123	171	Lactoferrin	3.26 (0.934)	293
Personal History of GI Disease	*N (Yes)*	*N (No)*	*Physiological Profile*	*Mean (SE)*	*Total N*
PH IBS	107	187	Copper/Zinc Ratio	1.452 (0.365)	277
PH Chrohns*	6	288	25-OH Vitamin D	38.951 (1.325)	270
PH Ulcerative Colitis*	4	290	Vitamin B-6	37.668 (2.284)	264
PH Peptic Ulcers*	7	287	Homocysteine	9.361 (0.274)	257
PH GERD	41	253	Urinary Histamine	20.722 (0.504)	248
PH Celiac*	11	283	Urinary Norepinephrine/ Epinephrine Ratio	5.649 (0.223)	253
PH Colon Cancer*	6	288	*Physiological Profile*	*N (Yes)*	*N (No)*
			Food Sensitivity	62	186

Family History of GI Disease	*N (Yes)*	*N (No)*	*Genetic Detoxification Profile*	*N (Yes)*	*N (No)*
FH IBS*	22	272	CYP1A	20	75
FH Chrohns*	7	287	CYP1B	83	12
FH Ulcerative Colitis*	3	291	NAT2*I114T	33	61
FH Peptic Ulcers*	4	290	NAT2*R197Q	52	42
FH Gerd*	4	290	*NAT2*G286E**	*3*	*91*
FH Celiac*	11	283	*NAT2*R64Q**	*0*	*94*
FH Colon Cancer*	18	276	NAT2*K268R	57	37
			COMT*V158M	77	18
Supplements	*N (Yes)*	*N (No)*	*Supplements*	*N (Yes)*	*N (No)*
Probiotics	215	73	Vitamin D	175	144
Zinc	127	162	Vitamin B6	125	164

For abnormal TSCFA status, 19 variables were found to be associated with a $p < 0.2$ (Table 2): male gender, age, marital status, having greater than a high school education, weight, sinus issues, alcohol consumption, taking zinc supplements, personal history of irritable bowel syndrome (PH_IBS) or gastroesophageal reflux (PH_GERD), symptoms of diarrhea, heartburn, stomach pain, or bloating, presence of yeast, sigA, or dysbiotic flora in stool, stool pH, methylation status, and abnormal copper/zinc ratio. Of these 19 variables associated with abnormally low TSCFA levels, graduate education, sinus issues, personal history of GERD, diarrhea, heartburn, stomach pain, fecal dysbiotic flora, fecal pH, and abnormal copper/zinc ratio had p-values <0.05. None of the other physiological variables nor the genetic detoxification profile variables were associated with abnormally low TSCFA levels.

Table 2. Bivariate relative risk regression for abnormal TSCFA status, predictors with $p < 0.2$.

Parameter	*Relative Risk*	*95% CI*	*P (Parameter Estimate)*
Gender (Male)	0.66	0.41-1.07	0.094
Age	1.01	1.00-1.02	0.135
Married (relative to single)	1.28	0.70-2.34	0.420
Widowed/Divorced (relative to single)	1.79	0.85-3.76	0.127
College Education (relative to HS only)	1.24	0.66-2.35	0.502
Graduate Education (relative to HS only)	1.53	0.82-2.86	0.178
Weight	1.00	0.99-1.00	0.143
Sinus Issues	1.61	1.09-2.38	0.017
Alcohol Consumption	0.71	0.47-1.07	0.102
Supplement: Zinc	1.32	0.89-1.97	0.168
Personal History of Irritable Bowel Syndrome	1.41	0.95-2.09	0.089
Personal History of Gastroesophageal Reflux Disease	1.68	1.07-2.62	0.023
Diarrhea	1.63	1.11-2.41	0.014
Heartburn	1.63	1.08-2.45	0.019
Stomach Pain	2.09	1.41-3.10	0.0003
Bloating	1.30	.088-1.93	0.192
Fecal Yeast	0.74	0.49-1.12	0.160
Fecal Dysbiotic Flora	0.46	0.25-0.82	0.009
Fecal Sig A	1.37	0.83-2.27	0.215
Fecal pH	2.82	1.98-4.01	<0.0001
Methylation Status	0.45	0.23-0.89	0.023
Copper/Zinc Ratio Abnormal	1.60	1.00-2.57	0.050

The first model examining predictors of abnormal total short chain fatty acid status excluded the physiological and detox genome markers, and failed to converge (Table 3). Given this failure of the first model using backwards stepwise regression, a forward selection model was developed using those predictors with the highest relative risk whose 95% CI did not include 1. This restricted model included the variables stomach pain – RR: 1.84 (1.22-2.76, $p = 0.004$) and fecal pH – RR: 2.36 (1.73-3.21, $p <0.0001$), both of which were significant predictors of abnormal

TSCFA status. Forward regression from this restricted model including additional variables with $p<0.2$ produced only one model with an additional significant predictor, methylation status.

Table 3. Multiple Logistic Regression models for TSCFA status.

Variables in model	*BIC*	*Relative Risk*	*95% CI*	*p*
Gender (Male)	--	*model failed to converge	--	--
Age				
Marital Status				
Single				
Currently Married				
Previously Married				
Education				
High School				
College Graduate				
Postgraduate				
Weight				
Sinus Issues				
Alcohol Consumption				
Supplements: Zinc				
PH_IBS				
PH_GERD				
Diarrhea				
Heartburn				
Stomach Pain				
Bloating				
Fecal Yeast				
Fecal Sig A				
Dysbiotic Flora				
Fecal pH				
Stomach Pain	287.615	1.84	1.22-2.76	0.004
pH	(N=271)	2.36	1.73-3.21	<0.0001
Stomach Pain	265.708	2.00	1.30-3.10	0.002
pH	(N=237)	2.10	1.50-2.95	<0.0001
Methylation Status		0.34	0.17-0.69	0.003

*Forward relative risk regression did not produce models with additional significant variables.

Table 4. Bivariate Relative Risk Regression Analysis for *Lactobacillus* status: Predictors ($p < 0.2$).

Parameter	*Relative Risk*	*95% CI*	*P (Parameter Estimate)*
Diarrhea	0.75	0.52-1.09	0.1289
Constipation	1.27	0.92-1.75	0.1435
Personal History of GERD	0.62	0.34-1.13	0.1151
Weight	1.00	1.00-1.01	0.1214
Vitamin B6 Abnormal	0.74	0.49-1.11	0.1453
Histamine Abnormal	0.79	0.56-1.11	0.1754
CYP1A Polymorphism	0.50	0.20-1.25	0.1395

Bivarable relative risk regression of predictors for *Lactobacillus* status revealed 7 variables with a $p<0.2$: diarrhea, constipation, PH_GERD, weight, abnormal B6 and abnormal histamine levels, and CYP1A polymorphism (Table 4). These predictors were used in further relative risk regression modeling. Because fewer subjects had results for the physiological and detox genome profile markers, the first model included only the symptom, patient history, and weight variables (Table 5). This full model produced no significant predictors of risk of abnormal levels of *Lactobacillus* in CSA. Permutations of models including only the GI symptoms as parameters also did not produce any significant predictors. When abnormal histamine and abnormal B6 are added to the full model, one significant predictor is found, weight ($p = 0.03$) However, with a relative risk of 1.00 (95% CI: 1.00-1.01), the actual clinical significance of this finding is questionable. The addition of the detoxification genome marker CYPIA to the model also did not produce additional significant predictors. In the bivariate logistic regression analysis of associations with abnormal *Lactobacillus* status, all of the 95% CI for the odds ratios included 1, indicating that there was likely no significant difference in *Lactobacillus* status by these predictor variables. Multiple relative risk regression analysis has born this out, with none of the models predicting *Lactobacillus* status well (c: 0.576-0.751).

Table 5. Multiple relative risk regression modeling for *Lactobacillus* status

Variables in model	*BIC*	*Significant variables*	*p*
Diarrhea Constipation PH_GERD Weight	368.120 (N=274)	None	–
Diarrhea Constipation PH_GERD	390.343 (N=294)	None	–
Constipation PH_GERD	386.789 (N=294)	None	–
Diarrhea Constipation	688.316 (N=294)		
Diarrhea PH_GERD	387.126 (N=294)		
Diarrhea Constipation PH_GERD Weight B6 Abnormal Histamine Abnormal	297.748 (N=213)	ns ns ns 1.00 (1.00-1.01) ns ns	 0.030
Diarrhea Constipation PH_GERD B6 Abnormal Histamine Abnormal	310.576 (N=226)	None	–
Diarrhea Constipation PH_IBS PH_GERD Weight B6 Abnormal Histamine Abnormal CYP1A	119.988 (N=77)	None	–

Variables in model	*BIC*	*Significant variables*	*p*
Diarrhea Constipation PH_IBS PH_GERD B6 Abnormal Histamine Abnormal CYP1A	119.095 (N=81)	None	–

For *Bifidobacter* status, 15 variables had a $p < 0.2$: College Education, Sig A, Dysbiotic Flora, Lactoferrin Abnormal, Supplements: B6, Diarrhea, Constipation, Food Sensitivity, Low Back Pain, Alcohol, B6 Abnormal, Methylation Status Abnormal, NAT2*K268R Polymorphism, NAT2*I114T Polymorphism, and CYP1A Polymorphism (Table 6).

Table 6. Bivariate Relative Risk Regression for *Bifidobacter* status

Parameter	*Relative Risk*	*95% CI*	*P (Parameter Estimate)*
College Education	1.31	0.91-1.89	0.1484
Sig A	0.84	0.67-1.05	0.1295
Dysbiotic Flora	1.27	1.02-1.57	0.0291
Lactoferrin Abnormal	1.35	1.09-1.67	0.0059
Supplements: Vitamin B6	1.15	0.93-1.43	0.1962
Diarrhea	1.19	0.96-1.48	0.1050
Constipation	0.84	0.67-1.05	0.1317
Food Sensitivity	1.20	0.94-1.53	0.1410
Low Back Pain	1.24	1.00-1.53	0.0540
Alcohol	1.20	0.91-1.59	0.1893
Vitamin B6 Abnormal	0.79	0.60-1.05	0.1036
Homocysteine Abnormal	0.85	0.68-1.08	0.1788
NAT2*K268R Polymorphism	1.63	0.96-2.75	0.0714
NAT2*I114T Polymorphism	1.73	0.98-3.06	0.0591
CYP1A Polymorphism	1.81	1.21-2.71	0.0040

Table 7. Multiple relative risk regression modeling for *Bifidobacter* status

Variables in model	*BIC*	*Relative Risk (95% CI)*	*p*
Education Dysbiotic Flora SigA Lactoferrin Abnormal Supplements: B6 Diarrhea Constipation Food Sensitivity Low Back Pain Alcohol	–	*model failed to converge	–
Lactoferrin Abnormal Dysbiotic Flora	389.847 (N=278)	1.24 (1.00-1.53) 1.35 (1.09-1.66)	0.0486 0.0051
Lactoferrin Abnormal Dysbiotic Flora Food Sensitivity	333.974 (N=235)	1.34 (1.06-1.66) ns 1.27 (1.01-1.58)	0.0127 0.0383
Lactoferrin Abnormal Food Sensitivity	338.750 (N = 239)	1.34 (1.06-1.67) ns	0.0143
Lactoferrin Abnormal Dysbiotic Flora NAT2*K268R NAT2*I114T CYP1A	–	*model failed to converge	–
Lactoferrin Abnormal NAT2*K268R NAT2*I114T CYP1A	132.137 (N=90)	1.74 (1.09-2.77) ns ns ns	0.0196
Lactoferrin Abnormal CYP1A	130.797 (N=91)	1.53 (0.97-2.43) 1.48 (0.97-2.25)	0.0699 0.0704
Lactoferrin Abnormal NAT2*K268R	126.936 (N=90)	1.78 (1.15-2.75) 1.65 (1.00-2.72)	0.0092 0.0492
Lactoferrin Abnormal NAT2*I114T**	125.773 (N=90)	1.83 (1.19-2.80) 1.80 (1.05-3.09)	0.0063 0.0336

**Forward regression from this model did not produce any additional significant predictors.

The first model examining predictors of abnormal *Bifidobacter* status excluded the physiological and detox genome markers, and failed to converge due to the relative Hessian convergence criterion being greater than the limit (0.0058 > 0.0001) (Table 7). Given this failure of the first model using backwards stepwise regression, a forward selection model was developed using those predictors with the highest relative risk whose 95% CI did not include 1. This revised model included the variables dysbiotic flora and abnormal lactoferrin level, both of which were significant predictors of abnormal *Bifidobacter* status. Forward regression from this restricted model identified food sensitivity –RR: 1.27 (1.01-1.58) as an additional significant predictor of abnormal *Bifidobacter* levels. Inclusion of food sensitivity in the model resulted in dysbiotic flora no longer being a significant predictor. However, a restricted model including abnormal lactoferrin and food sensitivity showed that food sensitivity was not a significant predictor of the outcome, after accounting for abnormal lactoferrin. Inclusion of the physiological markers to this model, individually and in combination, did not result in additional significant predictors. Adding the three detoxification genome markers (CYP1A, NAT2*K268R, NAT2*I114T) to the model including dysbiotic flora and abnormal lactoferrin levels again resulted in a model that failed converge due to the relative Hessian convergence criterion being greater than the limit of 0.0001. Excluding dysbiotic flora from this model including the genome markers showed only abnormal lactoferrin level to be a significant predictor of abnormal *Bifidobacter* status. Examining the detoxification genome markers individually in models including abnormal lactoferrin showed that all three are significant predictors of abnormal *Bifidobacter* status after controlling for abnormal lactoferrin, though the 95% CI for CYP1A includes 1, and the models looking at the contribution of NAT2*K268R and

NAT2*I114T both had lower BIC values (suggesting that they are better models for predicting abnormal *Bifidobacter* status).

DISCUSSION

This exploratory pilot study does not support the primary hypothesis that those with lower socioeconomic status (SES) would be more likely to have abnormal levels of total short chain fatty acids or beneficial gut bacteria. Only one variable related to socioeconomic status was available from the retrospective chart review, level of education. There was no information on income or occupation in the records, and the other proxy for SES (race/ethnicity) was only recorded for 89 patients, 84 of whom were White.

There is some suggestion from this study that chronic gastrointestinal diseases do impact TSCFA levels, as personal history of gastroesophageal reflux and irritable bowel syndrome showed a trend ($p < 0.2$) with abnormally low total short chain fatty acid levels. However, in the multivariable regression models, neither condition was significant in predicting abnormal TSCFA levels. Instead, abnormal methylation status showed a protective effect on abnormal TSCFA – RR: 0.34 (0.17-0.69, $p = 0.003$), after accounting for the effects of stomach pain and fecal pH. None of the components of the composite variable methylation insufficiency (i.e., abnormal histamine, abnormal homocysteine, and abnormal norepinephrine/epinephrine ratio) were associated with low TSCFA. Future work, using a larger sample size and healthy volunteers, should explore these components and examine if any particular one contributes more to abnormal levels of total short chain fatty acids.

None of the multivariable relative risk regression models were adequate in predicting abnormal *Lactobacillus* status. For *Bifidobacter,* abnormal lactoferrin levels, as well as several of the detoxification genome markers (CYP1A, NAT2 K268R, NAT2 I114T), showed a

significant increase in the risk for abnormal levels. Lactoferrin in stool is a marker of inflammation, and in a clinical context, can be used to distinguish patients with active inflammatory bowel disease from those with irritable bowel syndrome (Dai et al., 2007). The detoxification genes, CYP1A and NAT2, produce enzymes which are active in the breakdown of heterocyclic amines, potentially carcinogenic compounds produced from cooking meat (Huycke and Gaskins, 2004). CYP1A codes for a protein that belongs to the large cytochrome p450 enzyme family, which metabolizes steroid hormones and other fat-soluble molecules and oxidizes potentially toxic compounds (Liong, 2008). NAT2, a gene expressed primarily in the liver and GI tract, codes for n-acetyltransferase, an enzyme active in acetylation metabolic pathways. The K268R polymorphism signifies a fast metabolizer genotype, which may lead to mistakes in the acetylation process and failure to fully neutralize toxic compounds. The I114T polymorphism, in contrast, confers a slow metabolizer genotype, which could lead to the build up of toxic substances in the gut. Both NAT2 genotypes have been associated with increased risk of CRC, and the results of the present study suggest a possible interaction between host genotype and the intestinal microbiome (Frank et al., 2011). In addition, *Bifidobacter* species have been shown to bind to certain carcinogenic byproducts of heterocyclic amine metabolism, revealing a potential mechanism for such an interaction (Zhang and Ohta, 1993; Kulkarni and Reddy, 1994). Recent studies have also demonstrated differences in microbial profiles for those with colorectal cancer, who were more likely to have higher levels of *Bacteriodes/ Prevotella* than normal controls (Sobhani et al., 2011). While the comprehensive stool analysis reports in this retrospective chart review did not contain data on these particular species, preliminary results showing increased likelihood of low levels of one beneficial bacterial species, *Bifidobacter,*

associated with markers of inflammation and impaired detoxification pathways suggest intriguing possibilities for prospective studies.

The present study has several limitations. First, these data were collected as part of a retrospective chart review, from paper charts in an Integrative Medicine Clinic. During the abstraction process, it was noted that not all variables were available for all subjects. In addition, the comprehensive stool analysis reports were from two different laboratories, so many variables required recoding into normal/abnormal values based on the reference range of the particular lab, leading to loss of analytical information. Also, this population is distinct, being patients at the Integrative Medicine Clinic who, due to lack of reimbursement from insurance for physician visits, tend to be more affluent than patients from other specialties as they must pay many of the costs out-of-pocket. These patients also tend to be sicker than other groups, having exhausted many other treatment options before arriving at the Integrative Medicine Clinic. As a consequence, these results are not generalizable beyond this very specific group of patients, but do offer potential avenues for future research.

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