Supplementary Methods

Genotyping

Genotyping of 4 nonsynonymous SNPs in *KLB* and *FGFR4* with minor allele frequencies (MAF) >9% (Supplementary Table 1) were performed as in previous studies and summarized in the appendix. These coding SNPs lead to changes in the amino acid sequence of their corresponding protein and, therefore, to potential changes in protein function.

Genomic DNA was isolated from whole blood using standard methods shortly after blood draw and stored at −80°C until genotyping. Genotyping was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) per manufacturer's instructions. Following PCR amplification, end reactions were analyzed using ABI 7300FAST Real-Time PCR System by Sequence Detection Software (Applied Biosystems, Foster City, CA).

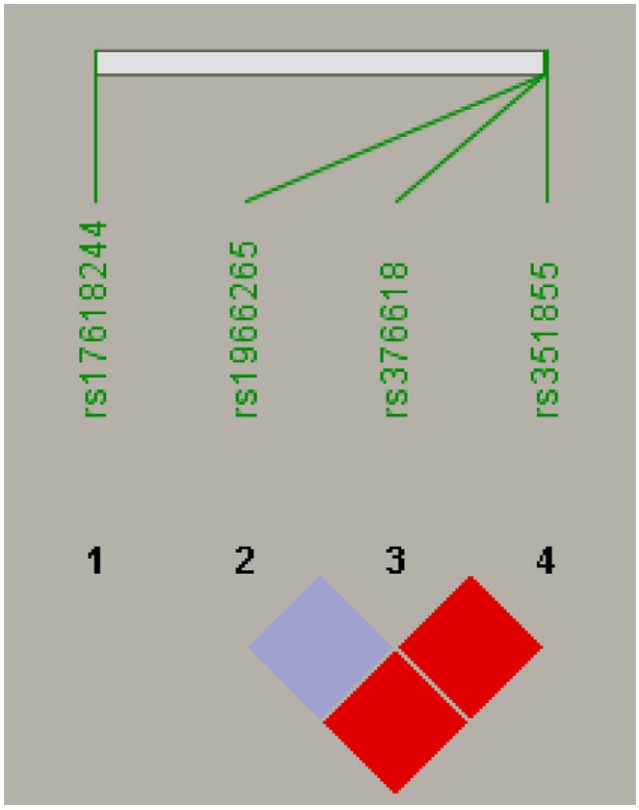
Supplementary Table 1. Candidate SNPs in *KLB* and *FGFR4* With *P* values for Hardy–Weinberg Equilibrium and MAF

					HV in full cohort n = 282		HV in transit cohort n = 56				
Gene	SNP rs ID	Major allele	Minor allele	Predicted MAF	Observed MAF	HWE *P* value	Observed MAF	HWE *P* value	Function	Amino acid position	Amino acid change
KLB	Rs17618244	G	A	0.155	0.181	.109	0.164	.325	Missense	728	Arg → Gln
FGFR4	Rs1966265	G	A	0.224	0.226	.309	0.200	.670	Missense	10	Val → Ile
FGFR4	Rs376618	T	C	0.225	0.204	.001*	0.273	.047*	Missense	136	Leu → Pro
FGFR4	Rs351855	G	A	0.283	0.333	.079	0.318	.210	Missense	388	Gly → Arg

HWE, Hardy–Weinberg Equilibrium.

NOTE. Predicted MAF is based on the HapMap-CEU population. Observed MAF is derived from genotypes of the healthy volunteers in the full study cohort of 714 subjects (n = 282) and the HV subset (n = 56) of the 238 subjects with transit measurements. HWE *P* value >.05 denotes that the study sample is in HWE for the corresponding SNP; the two *P* values not satisfying HWE are marked with an asterisk.

This table is reproduced from a more extensive table in Wong BS, et al. Gastroenterology 2011;140:1934–1942.[15]



Supplementary Figure 1. The 3 SNPS in FGFR4 were not in linkage disequilibrium.

Supplementary Table 2. Sample Size Assessment

Response	Observed overall mean	Observed (pooled) SD	Observed overall mean (log scale)	Observed (pooled) SD (log scale)	Effect sizes detectable with ~80% power at α = .05 (2-sided) n = 23/group: Log scale[a]	Effect sizes detectable with ~80% power at α = .05 (2-sided) n = 23/group: Geometric means[b]
Serum C4, *ng/ml*	28	27	2.909	0.984	25%	79%
Serum FGF19, *pg/ml*	130	95	4.681	0.589	10%	49%
48-h Stool bile acid, *μmol/24h*	682	801	6.054	0.989	13%	79%
48-h Stool fat, *g/24h*	5.3	5.5	1.443	0.716	35%	59%
48-h Stool weight, *g/24h*	132	104	5.284	0.824	12%	67%

NOTE. This table shows that the distributions for many of the responses were positively skewed; therefore, an assessment of the associations that could have been detected was based on first transforming to log scale. The associations corresponding to the differences (in log scale) are the *minimal* ones that could have been detected (with 80% power), and thus, for example, a clinically meaningful association corresponding to differences of 20% or 25% for stool weight could have been detected with somewhat greater power. The sample size of ~23 per group was expected to provide approximately 80% power (based on a 2-sample *t test* using a 2-sided α level of .05) to detect the effect sizes shown in Supplementary Table 2 for the end points of interest, ie, associations with group status corresponding to differences between pairs of groups for the end points of interest, eg, 12% (Log[stool weight]), 25% (Log[serum C4]), and 10% (Log[FGF19]).
[a]The effect size is 100* (the difference [in log scale]/the overall mean [in log scale]).
[b]The effect size is 100* (difference in geometric Means/mean of the geometric means).

Supplementary Table 3. Primary Measurements in 3 Subgroups

Data show median (IQR)	IBS-D	IBS-C	Healthy controls
Serum C4, *ng/ml*[a]	32.7 (18.9–68.6)	20.8 (5.7–30.6)	16.9 (6.1–28.0)
48-h Stool bile acid, *μmol/24h*[b]	863.5 (452.7–1213.1)	229.4 (141.5–596.7)	363.4 (193.7–762.1)
48-h Stool fat, *g/24h*	4.0 (3.0–8.0)	4.0 (2.0–4.0)	4.0 (2.0–7.0)

NOTE. Overall associations with subgroup: [a]$P = .02$; [b]$P = .057$.