# SUPPLEMENTAL MATERIAL

# Characterizing Factors Associated with Differences in FGF19 Blood Levels and Synthesis in Patients with Primary Bile Acid Diarrhea

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## Results: Further associations with genetic variants

A fuller description of the allelic and genotypic variants that were studied and the associations that were found is detailed below.

### FGF19

For the two SNPs in the 3’-UTR of the *FGF19* gene, no significant differences between the patient groups for allelic frequency and no dominance effects were found.

### FXR

The FXR variant rs61755050 produces a non-synonymous change (Met173Thr) and has previously been shown to affect FXR function. (1) Two subjects out of 111 successful genotypes were heterozygotes; both had severe PBAD (SeHCAT 3% and 2%), low serum FGF19 (59 and 149 pg/mL) with total fasting bile acids of 0.9 and 5.9 mmol/L respectively. This result was not significant due to small sample size; power calculations for a significance of 0.05 and a power of 0.80 suggest around 500 subjects would be required. The other FXR variant, rs56163822, which produces a change at the translation start site and has been associated with IBD and intrahepatic cholestasis of pregnancy, (1,2) has a low MAF and no significant difference was found with these small groups. One individual was homozygous for the minor allele but had ID with a SeHCAT value of 21% and a fasting FGF19 237 pg/mL.

### FGFR4

The *FGFR4* variant rs376618 has been linked to faster colonic transit in TT homozygotes. (3) We found no significant difference in MAF at a SeHCAT cut-off of 15%, but at lower cutoff values of 10% and 5%, the minor C allele was less frequent in PBAD than in ID patients, with uncorrected p values of 0.0127 and 0.0507 (Supplemental table 1).

When genotypes were looked at, there was a lower proportion of CT heterozygotes in PBAD compared with in ID subjects (24% vs. 43%) and CC + CT (i.e. C dominant) genotypes were also significantly less frequent compared to the TT genotype (p = 0.053). Proportions were significant at SeHCAT cutoffs of 10% and 5%. TT subjects have a significantly lower SeHCAT to those in the combined CT + CC group (11% vs. 19%, p = 0.04) but FGF19 levels were not significantly different (214 vs. 178 pg/mL, p = 0.33).

**Supplemental Table 1: Analysis of *FGFR4* SNP rs** **rs376618 at different SeHCAT values**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SeHCAT cutoff value | Minor allele frequency | |  | Genotype dominance model | |
|  | C | | T | C |
|  | ID | PBAD | p | p | p |
| 15% | 0.285 | 0.221 | 0.241 | 0.567 | 0.053 |
| 10% | 0.300 | 0.167 | 0.013 | 0.548 | 0.009 |
| 5% | 0.279 | 0.140 | 0.051 | 0.694 | 0.047 |

Idiopathic diarrhea (ID) patients have SeHCAT values greater than the cutoff and PBAD patients less than the cutoff value.

### KLB

The rs17618244 SNP in the *KLB* gene has previously been shown to be associated with colonic transit in IBS with the G allele also linked to faster gut transit time in response to oral chenodeoxycholate acid. (3,4) The G allele was more frequent in the PBAD group than in the ID group with p values about 0.1 for different SeHCAT cutoffs (see Supplemental Table 2).

The GG or GA genotypes were more common than the AA genotype but with only borderline significance at the SeHCAT cutoff of 15% (p = 0.07). Of the 8 subjects with the genotype AA, 7 had ID (SeHCAT ranging from 19 – 51 %), and only 1 had PBAD (SeHCAT 7%).

**Supplemental Table 2: Analysis of *KLB* SNP rs17618244 at different SeHCAT values**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SeHCAT cut-off value | Minor allele frequency | |  | Genotype dominance model | |
|  | A | | G | A |
|  | ID | PBAD | p | p | p |
| 15% | 0.188 | 0.120 | 0.118 | 0.072 | 0.365 |
| 10% | 0.183 | 0.106 | 0.098 | 0.270 | 0.181 |
| 5% | 0.173 | 0.077 | 0.096 | 0.353 | 0.224 |

Idiopathic diarrhea (ID) patients have SeHCAT values greater than the cutoff and PBAD patients less than the cutoff value.

### ASBT

The rs188096 SNP in the *SCL10A2* gene, encoding Ala171Ser in ASBT, has been found to be present in a family with congenital PBAD (5) and has been previously studied in larger groups of patients. (6) No significant difference was found here although the sample size is low. Three individuals had the AA genotype, one with ID (SeHCAT 37%) and two with severe PBAD (SeHCAT <2%). All 3 individuals were clinically otherwise unremarkable. The ID individual had an unremarkable FGF19 of 238 pg/mL and the two PBAD individuals also had atypical high FGF19 (260 and 313 pg/mL).

### OSTα

The rs939885SNP in OSTα had similar allelic frequencies in the two groups.

### GPBAR1

### The rs11554825 SNP in the 5’-UTR of the GPBAR1 gene has been associated with colonic transit and fecal bile acids in IBS patients (7) but we found no differences in allelic or genotype frequencies between our patient groups.

### TNFSF15

Although TNFSF15 is not involved with bile acids, SNPs including rs7848647 have been associated with post-infectious IBS and could have been increased in our ID controls. (8) No significant difference was found in our patient groups.

### Relationships with FGF19 phenotypic variation

In the 17 subjects where FGF19 was profiled throughout the day, allelic frequencies were compared between the L - L phenotype and the combined group of the other phenotypes. The FGFR4 SNP rs376618 showed a significantly higher proportion of the C allele in the L - L phenotype compared with the others (0.375 vs. 0.091, p = 0.05). The ASBT SNP rs188096 has a higher proportion of the A allele in the L-L phenotype (0.250 vs. 0.091, p = 0.22), and the FGF19 SNP rs948992 had a lower proportion of the G allele in the L - L group than the rest (0.188 vs 0.455, p = 0.17), but neither difference reached significance.

## Specific references

1. Van Mil SW, Milona A, Dixon PH *et al*. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. Gastroenterology 2007;133:507-16.

2. Attinkara R, Mwinyi J, Truninger K *et al*. Association of genetic variation in the NR1H4 gene, encoding the nuclear bile acid receptor FXR, with inflammatory bowel disease. BMC Res Notes 2012;5:461.

3. Rao AS, Wong BS, Camilleri M *et al*. Chenodeoxycholate in females with irritable bowel syndrome-constipation: a pharmacodynamic and pharmacogenetic analysis. Gastroenterology 2010;139:1549-58, 1558.

4. Wong BS, Camilleri M, Carlson PJ *et al*. A Klotho-beta Variant Mediates Protein Stability and Associates with Colon Transit in Irritable Bowel Syndrome with Diarrhea. Gastroenterology 2011;140:1934-42.

5. Oelkers P, Kirby LC, Heubi JE *et al*. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J Clin Invest 1997;99:1880-7.

6. Montagnani M, Love MW, Rössel P *et al*. Absence of dysfunctional ileal sodium-bile acid cotransporter gene mutations in patients with adult-onset idiopathic bile acid malabsorption. Scand J Gastroenterol 2001;36:1077-80.

7. Camilleri M, Shin A, Busciglio I *et al*. Genetic variation in GPBAR1 predisposes to quantitative changes in colonic transit and bile acid excretion. Am J Physiol Gastrointest Liver Physiol 2014;307:G508-G516.

8. Swan C, Duroudier NP, Campbell E *et al*. Identifying and testing candidate genetic polymorphisms in the irritable bowel syndrome (IBS): association with TNFSF15 and TNFalpha. Gut 2013;62:985-94.