Background

- Cholangiocarcinoma (CC) is increasing in incidence globally and its pathogenesis remains poorly understood.
- Chronic biliary inflammation and cholestasis are major risk factors for CC but most cases in the West are sporadic.
- Epidemiological studies have associated CC with various environmental toxins, hepatobiliary excretion is the main route of elimination of such toxins.
- Biliary transporter protein expression is variable and genetic polymorphisms in biliary transporters have been implicated in benign biliary disease.
- Progressive familial cholestasis type 2 (PFIC2) has been associated with childhood onset of CC.
- A recent case-control study of a single nucleotide polymorphism c.3972C>T in ATP8B1/CACT has suggested a potential association with CC.

Method

- DNA was obtained from 164 Caucasian patients with confirmed sporadic CC.
- A well matched control group was formed of 254 healthy Caucasian individuals with normal liver function tests.
- SNPs were selected using the HapMap database in Haploview 4.1 (MAF >0.05, pair-wise comparisons, R^2 cut-off of 0.8).
- 73 SNPs were selected, capturing the majority of common genetic variation around the five candidate loci (Table 1).
- Genotyping was undertaken with a competitive allele-specific PCR based robotic genotyping system (KasPar, Kbioscience, Herts, UK).
- Haplotype frequencies were compared using Haplo Stats v1.4.4.
- P values were corrected using the Bonferroni method and the false discovery rate (FDR) was calculated using QVALUE V1.0 in R V2.10.1.

Results

- All 73 SNPs were in Hardy-Weinberg Equilibrium.
- Four SNPs in ABCB11 were associated with altered susceptibility to CC, including the V444A polymorphism (c.1331T>C, rs2287622, p <0.007) (Table 3).
- One SNP in ABCB4 was associated with altered susceptibility to CC.
- Three SNPs in ATP8B1 were associated with altered susceptibility to CC.
- These 7 significantly associated SNPs did not retain statistical significance after Bonferroni correction for multiple testing.
- None of the SNPs in ABCB2 or NR1H4 showed association with CC.
- The calculated false discovery rate was 0.22, estimating 1.54 false positives in the 7 initially associated SNPs.
- Haplotype analysis of the genotyped SNPs in ATP8B1 identified significant differences in frequencies between cases and controls (global p-value 0.009).
- Haplotype analysis in ABCB11, ABCB4, ABCB2 and NR1H4 failed to detect any significant association.

Conclusion

- Largest study to date of biliary transporter polymorphisms and CC susceptibility.
- Reported association between SNP rs3740066 in ABCB2 and CC not replicated.
- Initially significant 7 individual SNPs did not survive Bonferroni correction.
- Haplotype analysis in ATP8B1 demonstrated a significant association.
- With less stringent correction for multiple testing (FDR), individual SNPs in each of ABCB11 and ATP8B1 suggest a potential association.
- Given the biological plausibility of polymorphisms in ABCB11 and ATP8B1 as risk modifiers for CC, further study in a validation cohort is required.

Acknowledgements:

A MM F

Imperial College Healthcare Charity

Supporting Mays and Hamlyn Research Hospitals.