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Genetic variation in biliary transporters as a susceptibility factor for cholangiocarcinoma

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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 and function determine bile flow, constituents and stability **(Figure 1)**
- Biliary transporter protein expression is variable and genetic polymorphisms in biliary transporter proteins 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 (rs3740066) in *ABCC2*, reported an association with CC

Aim

- To investigate five biologically plausible candidate genes as susceptibility factors for cholangiocarcinoma; *ABCB11* (BSEP); *ABCB4* (MDR3); *ABCC2* (MRP2); *ATP8B1* (FIC1) and *NR1H4* (FXR)

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 LFTs **(Table 1)**
- 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 2)**
- Genotyping was undertaken with a competitive allele-specific PCR based robotic genotyping system (KasPar, Kbioscience, Herts, UK).
- Confirmation of Hardy-Weinberg equilibrium and Cochran-Armitage trend testing were performed using PLINK v1.07 in R V2.10.1
- Haplotype frequencies were compared using Haplo Stats v1.4.4
- P values were corrected using the Bonferroni method
- 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 *ABCC2 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*, *ABCC2* 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 *ABCC2* 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

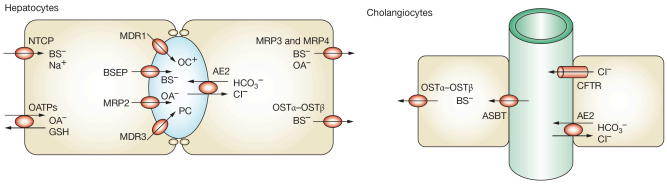

Figure 1 – Hepatocyte and cholangiocyte transporter proteins Abbreviations: ABCG5/G8; two-half transporter for cholesterol; AE2, chloride–bicarbonate anion exchanger isoform 2; ASBT, apical sodium-dependent bile-salt transporter; BCRP, breast cancer resistance protein; BS-, bile salts; BSEP, canalicular bile-salt export pump; CFTR, cystic fibrosis transmembrane regulator; Cl-, chloride ions; GSH, glutathione, HCO3-, bicarbonate ions; MDR1, multidrug export pump; MDR3, phospholipid export pump; MRPs, multidrug-resistance-associated proteins; MRP2, canalicular conjugate export pump; Na+, sodium ions; NTCP, basolateral sodium taurocholate cotransporter; OA-, anionic anions or conjugates; OATPs, organic anion transporting proteins; OC+, cationic drugs; OST, organic solute transporter; PC, phosphatidylcholine. (Adapted from Geier et al, 2006)

	n	Female (%)	Male (%)	Median age (range)
Controls	254	119 (46.8%)	135 (53.1%)	66.1 (55-80)
Cases	172	76 (44.2%)	96 (55.8%)	67 (30-92)

Table 1 – Demographic data of case and control groups

ABCB11 - Chromosome 2

Ref	RS number	BP location
1	rs497692	169497262
2	rs3755157	169500417
3	rs6709087	169507256
4	rs853773	169522593
5	rs853772	169522901
6	rs3821120	169525182
7	rs16823014	169525959
8	rs4148797	169526281
9	rs16856300	169526548
10	rs4148794	169529894
11	rs17267869	169531654
12	rs3770585	169532113
13	rs2287622	169538574
14	rs2058996	169542195
15	rs3770596	169548388
16	rs13416802	169550584
17	rs2287618	169551055
18	rs7605199	169564700
19	rs3815676	169578625
20	rs4148773	169582067
21	rs3814382	169597234
22	rs10930343	169598031
23	rs7577650	169599456

ABCB4 - Chromosome 7

Ref	RS number	BP location
1	rs31652	86867623
2	rs2097937	86868839
3	rs31653	86870549
4	rs2373593	86874878
5	rs31666	86890204
6	rs31668	86891142
7	rs8187799	86894112
8	rs31676	86907816
9	rs1149222	86911711
10	rs4148826	86912355
11	rs2109505	86917342
12	ABCB4_inde	86919500
13	rs1202283	86920228
14	rs2302386	86929880
15	rs4148812	86939343

ABCC2 - Chromosome 10

Ref	RS number	BP location
1	rs717620	101532568
2	rs2756109	101548736
3	rs4148391	101556856
4	rs2073337	101557416
5	rs2002042	101577921
6	rs7476245	101584719
7	rs3740066	101594197
8	rs3740065	101595683
9	rs3740063	101600713

NR1H4 - Chromosome 12

Ref	RS number	BP location
1	rs4764980	99409238
2	rs56163822	99411232
3	rs61755050	99450439
4	rs1030454	99469383
5	rs35724	99479509

ATP8B1 - Chromosome 18

Ref	RS number	BP location
1	rs7241054	53464481
2	rs1968274	53465209
3	rs17685876	53468119
4	rs317826	53474943
5	rs4308033	53485104
6	rs4306606	53485293
7	rs317845	53486410
8	rs317838	53489654
9	rs317837	53490222
10	rs11659313	53503387
11	rs319454	53504713
12	rs7236365	53509688
13	rs160993	53510410
14	rs319448	53511146
15	rs319440	53515528
16	rs17686300	53519745
17	rs319457	53519863
18	rs319406	53527210
19	rs319409	53529582
20	rs12456346	53546809
21	rs9676158	53555113

Table 2 – SNPs selected for genotyping by gene, RS number and location on chromosome

ABCB11/BSEP

RS number	Trend x^2	p	cor p	Allelic x^2	p	cor p
rs3770585	6.201	0.013	0.932	6.122	0.013	0.975
rs2287622	6.801	0.009	0.665	7.229	0.007	0.524
rs3770596	4.096	0.043	3.137	4.285	0.038	1.000
rs7605199	4.019	0.045	3.284	4.038	0.044	1.000

haplotype analysis

Global stat	df	p
14	9	0.120

ABCB4/MDR3

RS number	Trend x^2	p	cor p	Allelic x^2	p	cor p
rs2097937	3.924	0.048	3.475	4.045	0.044	1.000

Global stat	df	p
9.9	8	0.270

ABCC2/MRP2

RS number	Trend x^2	p	cor p	Allelic x^2	p	cor p
n/a						

Global stat	df	p
3.9	9	0.920

ATP8B1/FIC1

RS number	Trend x^2	p	cor p	Allelic x^2	p	cor p
rs319454	4.139	0.042	3.060	4.205	0.040	1.000
rs319448	4.576	0.032	2.367	4.852	0.028	1.000
rs12456346	3.686	0.055	4.005	3.916	0.048	1.000

Global stat	df	p
28	13	0.009

NR1H4/FXR

RS number	Trend x^2	p	cor p	Allelic x^2	p	cor p
n/a						

Global stat	df	p
8.8	8	0.360

Table 3 – Summary results for SNPs initially associated with altered susceptibility to CC showing χ^2 and p value pre- and post- Bonferroni correction and haplotype analyses results for each gene

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