was expressed by both the primary tumors and all the LCSCs and its blockage inhibited LCSC proliferation and migration in vitro. Conclusions. We established 6 cell lines from our model of hepatocholangiocarcinoma. Phenotype, tumorigenicity, and metastatic potential suggest that LCSCs harbor a population of G-CSFR+ cancer stem cells of OC origin. In the present study, we have also described the involvement of the G-CSF/G-CSFR axis in modulating LCSCs, and the inhibitory effects of G-CSFR blockage on LCSC proliferation and migration. These results may lead to new treatments against liver tumors, targeting stem cell-mediated cancer growth and preventing the establishment of metastases. Overall, LCSCs may be novel and useful cell lines for further studies of molecular pathways underlying hepatocarcinogenesis, as well as for the development of new cancer stem cell-targeted therapies.

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VARIABILITY OF THE ABCB4 GENE IN YOUNG ADULT CHOLECYSTECTOMIZED PATIENTS

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Aims and background: The ABCB4 (ATP-binding cassette, subfamily B (MDR/TAP), member 4) transporter expressed in the canalicular membrane of hepatocytes is responsible for phosphatidylcholine secretion into bile. Conceivably, variations of the ABCB4 gene could contribute to cholesterol gallstone formation through lowering biliary phosphatidylcholine secretion. The present study was undertaken to determine the frequency and type of ABCB4 genetic variability amongst patients < 40 years of age treated surgically for gallstone disease at a regional Norwegian surgical hospital. Materials and methods: One hundred and four patients (mean age 30 years, range 12-39) of Caucasian and Caucasoid ethnic background cholecystectomized on account of symptomatic cholesterol gallstone disease were included in the study. Genomic DNA from peripheral white blood cells was extracted on MagNApure LC from Roche. Coding exons 2 to 28 in the ABCB4 gene, (RefSeq NM_000443.3) including exon-intron boundaries were sequenced using an Applied Biosystem 3730 DNA Analyzer using Big Dye Terminator v.3.1 Sequencing kit. Predictions of functional consequences of protein-altering variants were assessed by amino-acid substitution matrices (Grantham, BLOSUM62) and computational tools that utilize position-specific evolutionary properties of the protein (SIFT, PolyPhen). Results: 12 exon variations were found, 8 of which were protein-altering: 6 missense, 1 frameshift and 1 nonsense. 11 intron variations were found. Of the 8 protein-altering variations 5 were predicted to have functional consequences (c.523A>G, c.1769G>A, c.3318G>C, c.1399_1400 ins10, c.3136C>T). Conclusion: Substantial genetic variability exists amongst young Caucasian and Caucasoid patients < 40 years of age cholecystectomized at a Norwegian regional hospital. The ABCB4 genetic variations: c.523A>G, c.1769G>A, c.3318G>C, c.1399_1400 ins10, c.3136C>T may contribute to an early debut of cholesterol gallstone disease.

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HLA ASSOCIATION IN PRIMARY SCLEROSING CHOLANGITIS: DETECTION AND FINEMAPPING OF AN HLA INDEPENDENT SIGNAL IN THE COMPLEMENT GENE CLUSTER

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Background: An association between the risk of developing primary sclerosing cholangitis (PSC) and genetic variants within the HLA complex on chromosome 6p21 was detected 25 years ago. This genetic region contains more than 250 closely linked genes, and pinpointing the genetic variants of relevance to PSC has proven extremely difficult. In an attempt to further refine the HLA association in PSC, systematic mapping of the entire HLA complex was performed in a large cohort of Scandinavian PSC patients. Materials and methods: 365 PSC patients and 368 healthy controls were genotyped for all classical HLA loci (HLA-A, -B, -C, DRB1, DRB3 and DQB1) using a sequencing based approach. A two-stage screen using single nucleotide polymorphism (SNP) markers was subsequently performed. In stage one, 420 SNPs were successfully genotyped with SNPlx® technology. Dissection of the association signals was performed using established statistical packages as well as novel statistical approaches. In stage two, saturation of a distinct risk region using additional 130 SNPs was performed to localize causative variants. Results: The SNP screen revealed the presence of a wide and complex association signal blurred by a strong LD haplotype that may harbor several variants strongly associated with PSC, e.g. at HLA-B (odds ratio [OR] HLA-B*08 = 3.5, 95% CI [2.6-4.5], p<10^-16) and MICA (OR MICA*006_A = 3.5, 95% CI [2.7, 4.5], p<10^-16), which have also been reported in previous studies. When case haplotypes were proactively matched with randomly drawn control haplotypes at markers defining this strong LD haplotype (i.e. HLA-B*08 and DRB1*0301), a distinct association signal became evident at the complement factor gene cluster within the central HLA class III region. Maximum association in this risk region was observed for a common allele at an intronic SNP (69% vs. 48%, OR=2.5, 95% CI [2.0, 3.1], p=10^-16). Interestingly, this allele maps to all known HLA risk haplotypes in PSC (i.e. DR3, DR6 and DR2), while previously reported protective HLA haplotypes in PSC (i.e. DR4, DR7 and DR11) predominantly carry the opposite allele at this position. Conclusion: The present dataset provides an extensive insight into the complexity of the HLA association in PSC. Multiple risk variants are likely to exist, some of which have been previously reported in other autoimmune diseases (e.g. at HLA-B and MICA), and others which are PSC-specific. Novel alignment strategies with known risk and non-risk HLA haplotypes in PSC helped to dissect a distinct risk locus for PSC in the central HLA class III region. Ongoing analyses aim to identify the exact susceptibility gene within this region.
THE NATURAL HISTORY OF SMALL-DUCT PRIMARY SCLEROSING CHOLANGITIS

Einar Bjorcnson, Rolf Olsson, Annika Bergquist, Stefan Lindgren, Barbara Braden, Roger Chapman, Kirsten Boberg, Paul Angulo

Background: Previous studies suggested that patients with small-duct primary sclerosing cholangitis (PSC) have a favorable prognosis in comparison to those with large-duct PSC. Prior studies, however, have included limited numbers of patients with a relatively short follow-up, with minimal or no power for an appropriate analysis of long-term survival. Hence, we aimed at determining the natural history and long-term prognosis of a large number of patients with small-duct PSC and to compare the outcomes and survival to that seen in appropriately matched patients with classic large-duct PSC.

Methods: Data from 83 patients with well-characterized small-duct PSC from several medical institutions in Europe and the United States were combined. Each patient with small-duct PSC was randomly matched to two patients with well-characterized large-duct PSC by age, gender, calendar year of diagnosis, and institution. Outcomes included development of cholangiocarcinoma (CCA), need for liver transplantation, and death. The Kaplan-Meier product limit was used for estimating survival free of liver transplantation.

Results: The median age at diagnosis in both groups was 38 years with 61% being males. Cases of small-duct PSC had a significantly longer survival free of liver transplantation as compared to controls with large-duct PSC (p<0.0001, logrank test). The median follow-up was 13 years (interquartile range 10-17) in the large-duct PSC group and 10 years (interquartile range 6-14) in the large-duct PSC group. Nineteen (22.9%) out of the 83 small-duct PSC patients progressed to large-duct PSC as verified by cholangiography. One patient with small-duct PSC who progressed to large-duct PSC was diagnosed with CCA but after large-duct PSC had been identified; 20 patients in the large-duct PSC group developed CCA during follow up. The number of deaths and liver transplant procedures performed were 11 and 8 respectively in the small-duct PSC group, and 45 and 33 respectively in the large-duct PSC group. The proportion of patients dying and/or undergoing liver transplantation was higher in the large-duct than small-duct PSC group (49.7% vs. 25.7% respectively, p<0.0001). Interestingly, two patients originally transplanted for end-stage small-duct PSC developed recurrent small-duct PSC in the graft requiring re-transplantation 9 and 13 years later. Conclusions: Patients with small-duct PSC have significantly better long-term survival than patients with large-duct PSC. Almost a fourth of patients with small-duct PSC progress to large-duct PSC over an average of 13 years of follow-up. Cholangiocarcinoma does not seem to occur in patients with small-duct PSC.

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