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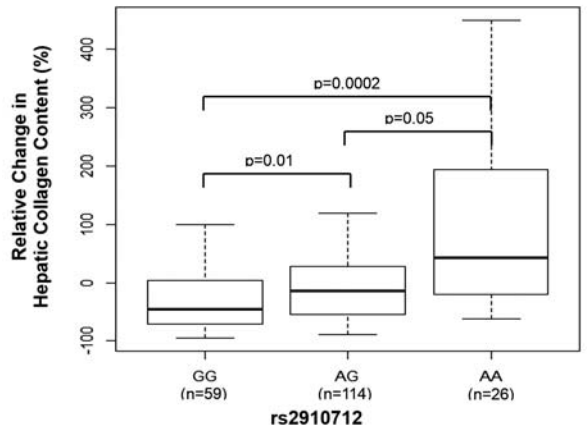
Background and Aims: Primary sclerosing cholangitis (PSC) is characterized by progressive fibrosis of the hepatobiliary tree that may lead to end-stage liver disease. Factors that influence fibrosis progression are poorly understood. In order to determine whether common human genetic variation influences PSC-related fibrosis progression, we performed a genome-wide association study (GWAS) of longitudinal changes in fibrosis endpoints in a phase 2b clinical trial.
Methods: We genotyped PSC patients enrolled in a phase 2b, placebo-controlled trial of simtuzumab (SIM), a monoclonal antibody against lysyl oxidase-like-2. Since SIM was ineffective in this trial, study groups were combined for this analysis. High-quality genotyping data were obtained from 200 patients for over 2 million single nucleotide polymorphism (SNP) loci using the Illumina Omni5 high density BeadArray chip. GWAS were conducted using linear or logistic regression of the change in fibrosis endpoints over 96 weeks including hepatic collagen content by morphometry, Ishak fibrosis stage, progression to cirrhosis, and PSC-related clinical events (e.g. hepatic decompensation, ascending cholangitis, cholangiocarcinoma, liver transplantation). All analyses were Bonferroni adjusted to correct for multiple testing and included age, gender, race, and BMI as covariates.
Results: At baseline, the median age was 45 years, 64% were male, 86% were Caucasian, and 47% had ulcerative colitis. 53% of the patients had bridging fibrosis or cirrhosis and the median (IQR) hepatic collagen content was 4.5% (IQR 2.7–7.0%). After correcting for multiple testing, we identified a genome-wide significant association ($p = 2.29 \times 10^{-8}$; linear regression) between the percent change in hepatic collagen from baseline to week 96 and common variation on chromosome 5, near the *GDNF* locus (rs2910712; Figure). This locus has previously been associated with a variety of chronic inflammatory and fibrotic disorders. The observed association was slightly attenuated after adjustment for baseline fibrosis stage ($p = 1.12 \times 10^{-7}$). No SNPs exceeded the genome-wide threshold for statistical significance for changes in Ishak stage, progression to cirrhosis, or PSC-related clinical events.

Figure: Relative Change in Hepatic Collagen Content between Baseline and Week 96 According to the rs2910712 SNP near *GDNF*



Conclusions: Our GWAS of the longitudinal progression of fibrosis-related traits among patients with PSC has identified a novel, genome-wide significant association with the change in hepatic collagen content over 96 weeks. Additional characterization of the GDNF association and functional assessment may provide insight into its role in liver fibrosis progression in PSC.

PS-003
IgG4 and IgG1 autoantibodies against Annexin A11 in IgG4-related disease of the biliary tract and pancreas

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Background and Aims: Immunoglobulin G4-related disease is a rare immune-mediated syndrome involving various organs, including the pancreas (autoimmune pancreatitis) and biliary tract (IgG4-associated cholangitis). Patients are characterized by elevated IgG4 serum levels and affected tissues typically show infiltrating IgG4-producing B and plasma cells. IgG4 antibodies possess certain biological properties that are considered anti-inflammatory, but the role that IgG4 plays in IgG4-related disease remains enigmatic. Recently, we identified a limited number of highly expanded IgG4 +B-cell receptor clones in blood and affected tissue of patients with active IgG4-related disease of the pancreas and biliary tract, suggesting that specific antigenic stimuli are involved. This research project aimed to identify possible target (auto) antigens for IgG4 that may be involved in the immune response in IgG4-related disease.
Methods: We screened sera of patients with IgG4-associated cholangitis and/or autoimmune pancreatitis (n = 44), for reactivity against a H69 cholangiocyte cell line lysate on immunoblot. Subsequently, IgG4-antigens were immunoprecipipated using IgG4-capturing beads and identified by quantitative mass-spectrometry. Sera of patients with primary sclerosing cholangitis (n = 18) or pancreatobiliary malignancies (n = 24) were used as controls.
Results: We found that IgG4, but also IgG1, from sera of patients with IgG4-related disease of the biliary tract and pancreas (n = 7), but not of controls, showed reactivity against a ±56 kDa cytosolic protein. Peptide mass fingerprinting identified Annexin A11, belonging to the Annexin family of calcium-dependent phospholipid binding proteins. The specificity of the patient sera for Annexin A11 was confirmed by screening reactive sera against an Annexin A11 knockdown cell lysate on immunoblot and against recombinant Annexin A11 in an enzyme-linked immunosorbent assay.
Conclusions: IgG1 and IgG4 autoantibodies against Annexin A11 may be involved in initiating and/or maintaining the immune response in IgG4-related disease of the biliary tract and pancreas.

PS-004
Improvement of gene therapy for Wilson's disease

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Background and Aims: Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism caused by mutations in the *ATP7B* gene that result in abnormal accumulation of copper in the liver, the cornea and the brain. Excessive copper accumulation leads to progressive damage in these organs and results in multisystemic manifestations. Current therapies for Wilson's disease are based on life-long treatments which may cause severe side effects and do not restore normal copper metabolism. We have recently demonstrated that the administration of an AAV vector expressing *ATP7B* under the control of a liver specific promoter into Wilson disease mice induces full restoration of copper homeostasis. However, the size of the vector genome surpasses the optimal size of

a packaged AAV genome difficulting its production and preventing the use of bigger promoters or additional regulatory sequences.
Methods: In the present work we have designed two truncated versions of the ATP7B protein and analyzed their functionality and therapeutic efficacy *in vivo*. ATP7B protein contains 6 metal binding sites (MBS) in its N-terminal region. It has been described that some of these sites can be deleted without compromising the functionality of the protein. With this in mind, we eliminated different fragments of the N-terminal region of the protein in order to generate shorter versions of ATP7B. To produce the truncated version 1 (T1) we eliminated 1 MBS and a fragment of the adjacent one; while in the truncated version 2 (T2) we depleted the first 4 MBS.
Results: The administration of recombinant AAV vectors carrying the truncated proteins under the control of a liver specific promoter to Wilson disease mice showed that both variants were able to reduce liver damage, copper in the urine and in the liver confirming their functionality. However, while the T2 variant was as efficient as the full-length version of ATP7B protein, T1 retained only a partial effect. Surprisingly, T2 was more efficient than wild type (WT) ATP7B protein in restoring ceruloplasmin oxidase activity in serum and showed therapeutic efficacy even when administered to mice with an advanced disease state, situation in which the genetic transfer of WT ATP7B showed no benefit.
Conclusions: In conclusion, we have identified a fully functional truncated version of ATP7B protein that will allow reducing the size of the therapeutic AAV vector facilitating its production and may show higher therapeutic potential than the WT ATP7B protein.

PS-005
Ductular reactions provide a structural biliary network to preserve bile drainage in the CDE-model of hepatocellular chronic liver injury

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Background and Aims: Ductular reactions (DR) are observed in many acute and chronic liver diseases in both humans and rodents. Previous studies demonstrated that DR could differentiate into hepatocytes and cholangiocytes *in vitro*, suggesting that DR act as a reservoir, activated following severe or chronic injury, generating the two epithelial liver cells to restore hepatic cell loss. However, only a small number of hepatocytes were found to be formed from DR cells *in vivo* and only in the choline deficient diet supplemented with ethionine (CDE)-model of chronic hepatocytic liver injury. This observation prompted us to investigate the pathophysiological role of DR *in vivo* in the CDE model. We propose that chronic hepatocellular injury causes a disruption of the canalicular bile flow and that DRs expand as a biliary structure that connects to hepatocytes in order to restore bile drainage from canaliculi to ductules.
Results: In this study, we demonstrate that intralobular bile flow is impaired following CDE liver injury, leading to distortion of the canalicular morphology with disruption of the tight junction integrity and luminal dilation of bile canaliculi in pericentral hepatocytes. We then show that DR cells share functional characteristics of cholangiocytes (expression of mucin-1, secretin receptor and CFTR), are organized around a tubular lumen lining on a basement membrane and possess the machinery needed to sense bile (primary cilium).
Conclusions: In conclusion, our results support that, in the CDE-model of hepatocellular chronic liver injury, DRs expand and penetrate into the parenchyma as linking biliary-like cells able to establish connection with hepatocytes to channel bile from canaliculi to ducts, thereby ensuring bile drainage in the injured and healing liver.

PS-006
MAdCAM-1 expression is absent in the hepatic tissue of paediatric autoimmune liver disease with associated inflammatory bowel disease

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Background and Aims: Autoimmune Liver Disease (AILD) disease is the classical extra intestinal manifestation of inflammatory bowel disease (IBD). The most common autoimmune hepatobiliary associations are primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). The discovery that mucosal addressin cellular adhesion molecule 1 (MAdCAM-1) is aberrantly expressed in the hepatic endothelium of adult PSC patients provided suggestive evidence that mucosal lymphocytes are recruited to the liver in AILD-IBD patients mediated via β7/MAdCAM-1 interactions. We aimed to further investigate this aetiopathogenic hypothesis in the juvenile form of PSC commonly known as autoimmune sclerosing cholangitis (ASC) and juvenile AIH with concomitant IBD.
Methods: Matched liver and gut (AILD-IBD n = 11 [ASC n = 6, AIH-I n = 5], AILD without bowel disease n = 2) biopsy specimens from AILD patients were formalin-fixed, paraffin embedded. Control liver tissue was sourced from both genetic and viral chronic liver diseases (HBV n = 6, HCV n = 6, Wilson's Disease n = 6) with control gut tissue sourced from IBD without liver disease patients (n = 6). All slides were stained with anti-CD3 and anti-MAdCAM-1 primary antibodies. MAdCAM-1 expression was assessed as either positive or negative and CD3 expression was quantified morphometrically.
Results: MAdCAM-1 expression was present in the colonic tissue of AILD-IBD patients and IBD patients without liver disease; however, there was no expression of MAdCAM-1 found within the liver tissue of the AILD-IBD patients. Furthermore, MAdCAM-1 expression was absent in the hepatic tissue of AILD without bowel disease and both genetic and viral chronic liver disease control tissue. There were no differences in the number of CD3+ cells present within the hepatic tissue of AILD-IBD and control liver tissue.
Conclusions: (1) We have shown that lymphocyte, namely CD3+ T cell, infiltration is a feature of juvenile AILD-IBD comparable to other chronic liver disorders; (2) Juvenile AILD-IBD may possess a differing aetiology to adult AILD-IBD that does not conform to the hypothesis that mucosal lymphocytes are recruited to the liver initiating and perpetuating hepatic autoimmunity. Previous findings in adult studies, specifically in PSC cohorts, have given efficacy for the potential use of anti-β7 integrin antibodies as a novel therapy for the treatment of AILD-IBD; however, these data suggest that such therapies could prove ineffective in the treatment of juvenile AILD-IBD.

PS-007
Ileo-anal pouch anastomosis negatively impacts graft survival following liver transplantation for primary sclerosing cholangitis

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Background and Aims: Liver transplantation is the only life-extending intervention for patients with primary sclerosing cholangitis (PSC). Given the co-existence of colitis with PSC and heightened