4th International Symposium on Inherited Diseases of the Pancreas

November 7–9, 2003, Chicago, Ill., USA

Selected Abstracts

Guest Editors
Randall E. Brand, Chicago, Ill.
Markus M. Lerch, Greifswald
David C. Whitcomb, Pittsburgh, Pa.
1 Psychosocial Impact of Hereditary Pancreatitis, and of Participation in the EURO PAC Research Register

I. H. Ellis\textsuperscript{1}, F. McDonald, J. P. Neoptolemos, L. Kerzin-Storrar
\textsuperscript{1}EUROPAC Study, Department of Clinical Genetics, Alder Hey Children’s Hospital, Liverpool, UK

This study followed up EURO PAC patients with hereditary pancreatitis (HP), to document the psychosocial impact of their condition and participation in research. 17 patients with identified PRSS1 mutations and who had received genetic counseling through EURO PAC were approached. 11 patients completed a questionnaire; six of these underwent semi-structured interview. Areas examined included diagnosis, information, support and attitudes to research. Data interpretation employed thematic analysis (qualitative) and non-parametric statistics (quantitative). Long delays in diagnosis of pancreatitis (mean 13.2 years) were common, and contributed to problems in doctor-patient relationships. Both diagnosis of pancreatitis, and molecular genetic diagnosis of HP, were therefore felt to provide validity. Patients’ main needs prior to EURO PAC were information and support. Patients received significantly more information from EURO PAC than from their own doctors (p < 0.05), but not significantly more support, suggesting a possible role for a lay support group. Disclosure of pancreatic cancer risk was seen as appropriate by 10 of 11 patients, and had little impact on worry among patients previously unaware of their risk. We conclude that HP patients benefit from participation in research, and can be helped by diagnosis, accurate information, genetic counseling, and patient-centered medical management.

2 Restoration of CFTR Function by Gentamicin in Cystic Fibrosis Patients Carrying Stop Mutations: A Double Blind Placebo Controlled Trial

Hadassah Medical Organization, Jerusalem, Israel

Background: Mutations in the cystic fibrosis conductance regulator (CFTR) gene containing a premature termination signal produce little or no functional chloride channel activity. Aminoglycoside antibiotics can suppress premature termination codons, thus permitting translation to continue to the normal termination of the transcript.

**Objectives:** To determine if topical administration of gentamicin to the nasal epithelium of patients with cystic fibrosis (CF) could result in expression of functional CFTR channels.

**Methods:** In a double-blind placebo controlled crossover trial, CF patients carrying stop mutations or patients homozygous for the C4F508 mutation received nasal drops containing gentamicin (0.3%, 3 mg/ml) 2 drops three times daily or placebo, for 2 consecutive periods of 14 days. Nasal potential difference (PD) was measured at baseline and after each of the 2 treatment periods. Nasal epithelial cells were obtained from CF patients carrying stop mutations, and surface CFTR was stained before and after gentamicin treatment, using the monoclonal 24-1 antibody which recognizes amino acids at the C-terminal of CFTR protein.

**Results:** Gentamicin treatment caused significant reduction of the basal PD in the 19 patients carrying stop mutations from ~45 B1 8 mV to 34 B1 11 mV (p < 0.005) and a significant response to chloride-free + isoproterenol solution from 0 B1 3.6 mV to ~5.2 B1 2.7 mV (p < 0.001). This effect of gentamicin on nasal PD occurred in both homozygous and heterozygous patients for stop mutations, but not in C4F508 homozygous patients. Following gentamicin treatment, a significant appearance of peripheral and surface CFTR staining was observed in the nasal epithelial cells of patients carrying stop mutations.

**Conclusions:** In CF patients carrying premature stop codons, gentamicin can cause translational read-through, resulting in CFTR protein localized at the apical cell membrane and thus correct the typical electrophysiological abnormalities caused by CFTR dysfunction. Further demonstration of the clinical effect of gentamicin may lead to the development of stop mutation specific therapy aiming to readthrough the genetic defect. This may result in useful therapy for CF and other genetic diseases caused by premature stop codons.

3 The R122H Cationic Trypsinogen Gene Mutation in Two Polish Families with Hereditary Pancreatitis

B. Korchowski, R. Mountford, J. P. Neoptolemos, I. H. Ellis
University of Rzesz, Rzesz, Poland

**Abstract:** Hereditary pancreatitis (HP) is a rare autosomal dominant genetic condition characterized by recurrent episodes of pancreatic attacks, frequent progression to chronic pancreatitis and high risk of pancreatic cancer. Mutations in the cationic trypsinogen gene are responsible for synthesis of altered structure proenzyme. Mutant trypsinogen is inappropriately activated inside pancreatic acinar cells, resulting in gradual pancreatic autodigestion and subsequent pancreatitis. The penetrance of mutations is approximately 80% with great variability within and between families. Two large,
Background: Human pancreatic cancer (PaCa) cells respond to multiple GPCR agonists, including bombesin, bradykinin, CCK, and neurotensin (NT). In particular, all K-Ras mutated PaCa cell lines examined express functional NT receptors [Ryder NM, Guha S, Reber HA, Rozengurt E, et al: J Cell Physiol 2001;186:53–64]. We also demonstrated that NT promoted clonal proliferation of PaCa cells. Thus, broad-spectrum neuropeptide antagonists including substance P (SP) analogues (e.g. [DArg1,DTrp5,7,9,Leu11]SP) [SPA], could provide a novel therapeutic approach for PaCa.

Aim: We will determine the effect of SPA on NT-induced mitogenic signaling, cell proliferation and growth of a PaCa xenograft in nude mice.

Methods and Results: SPA significantly blocked NT-induced transient increase in [Ca2+]i in PaCa cells, including PANC-1, MIA PaCa-2 and HPAF-II. Recently, we showed that NT potently stimulates ERK activation in PANC-1 [Guha S, Rozengurt E, et al: Cancer Res 2002;62:1632–1640 and 2003;63:2379–2387]. Consequently, SPA abrogated NT-induced MAPK activation in PaCa cells in a dose-dependent manner. Next, we determined the effect of SPA on PaCa cells growing on polyhydroxyethylmethacrylate coated dishes with no attachment to the substratum. SPA significantly reduced (by 45%) the anchorage-independent growth of PaCa, a hallmark of neoplastic progression. Finally, we examined whether SPA could inhibit PaCa growth in vivo using an HPAF-II xenograft in nude mice. Treatment was started when the tumors reached a mean diameter of 5 mm and the treatment was continued daily for 10 days. No observed toxicity or weight loss was noted in nude mice receiving 35 μg/g/day dose of SPA. We detected 40% decrease in tumor volume in the treated group, which is statistically significant (p < 0.05, student’s t-test). This marked growth inhibition was maintained at least two weeks beyond the end of the antagonist treatment.

Conclusions: We showed that the broad-spectrum neuropeptide antagonist, [DArg1,DTrp5,7,9,Leu11]SP, potently inhibited signal transduction pathways in vitro and significantly delayed the growth of a PaCa xenograft in vivo. These results, for the first time, support the hypothesis that the SPA analogues could be useful anti-proliferative agents against PaCa. Furthermore, this provides the basis for novel non-cytotoxic combination therapeutic strategies in PaCa.
Pilot Study BRCA2 and CDKN2A Mutations among Pancreatic Cancer Cases in the Northwest Cancer Genetics Network

M.A. Austin, D.J. Bowen, W. Burke, A. Fishbach, M.D. Fesinmeyer, J.D. Potter

University of Washington and Fred Hutchinson Cancer Research Center, Seattle, Wash., USA

Background: Previous studies suggest that germline mutations in the BRCA2 gene and the CDKN2A gene, that encodes the p16 protein, may be involved in susceptibility to pancreatic cancer, especially in familial forms of cancer. However, this association has not been studied in a population-based sample of pancreatic cancer cases.

Objectives: To determine the prevalence of BRCA2 and CDKN2A mutations among pancreatic cancer cases with a family history of pancreatic cancer, breast cancer or melanoma in the population-based Northwest Cancer Genetics Network (NWCGN).

Methods: Eighteen NWCGN participants reporting pancreatic cancer and an appropriate family history of cancer were identified based on interview data. Each case, or his/her next-of-kin if case was deceased, provided informed consent to use DNA samples for BRCA2 or CDKN2A genetic testing performed at Myriad Genetics. Pre- and post-test genetic counseling was provided to each participant.

Results: Of the 6 cases with a 1st or 2nd degree family history of pancreatic cancer, no mutations in the BRCA2 gene were found, and 3 of these cases had smoked at least 100 cigarettes during his/her lifetime. To date, 5 of these cases have been tested for CDKN2A mutations, and none was found. Of the 9 pancreatic cancer cases with only a family history of breast cancer, no BRCA2 mutations were found, although two of these cases reported islet cell cancer and two others had variants of unknown significance (V2969M, S384F). Among the 3 cases with a family history of melanoma, no mutations in the CDKN2A gene were found. All pancreatic cancer cases with either a family history of breast cancer or melanoma had a history of smoking.

Conclusions: In this population-based sample of pancreatic cancer cases with a family history of pancreatic cancer, breast cancer or melanoma, no deleterious mutations in the BRCA2 or CDKN2A gene have been found. Although the sample size of this pilot study is limited, the findings suggest that mutations in these genes are rare among pancreatic cancer cases, even among those with a family history of cancer. Thus, other genetic, environmental and behavioral factors, including smoking, must be involved in familial susceptibility to this disease.

Prophylactic Magnesium Treatment Attenuates the Onset and Course of Acute Pancreatitis


Department of Medicine B, University of Münster, Department of Medicine A, University of Greifswald, Greifswald, Germany

Background: Magnesium deficiency is a well documented phenomenon in patients with acute pancreatitis whereas hypercalcaemia is a well known risk factor for the onset of pancreatitis.

Objectives: Since we have shown that magnesium can act as an intracellular calcium antagonist we tested the hypothesis that its dietary supplementation could have a beneficial effect on the course of experimental pancreatitis in an animal model.

Methods: Four groups of male Wistar rats received diets containing different amounts of magnesium (<300 ppm, 450 ppm, 1,950 ppm and 30,000 ppm) for two weeks. Acute pancreatitis was then induced by infusion of supramaximal concentrations of caerulein (10 μg/kg/h) and control groups received either a physiological concentration of caerulein 1 μg/kg/h, 0.1 μg/kg/h or NaCl 0.9% for 4 h. In serum and plasma we determined amylase and lipase activity as well as calcium and magnesium concentrations. Trypsinogen activation peptide (TAP) was determined in urine and pancreatic tissue. Pancreatic tissue was used for EM-morphology and to determine trypsin and elastase activities. Pancreatic edema was measured as wet/dry weight ratio after Desiccation (160°C, 12 h).

Results: The magnesium content in serum increased significantly under the magnesium-enriched diets whereas serum calcium levels increased under magnesium-depleted diet. Amylase and lipase activities, pancreatic edema and the number of cytoplasmic vacuoles per cell in response to supramaximal caerulein were significantly reduced in rats fed a magnesium enriched diet. A significant increase in urinary and pancreatic TAP as well as increased trypsin and elastase activities were found in animals fed the magnesium depleted diet – not only in response to supramaximal but also in response to physiological caerulein concentrations.

Discussion: Oral magnesium supplementation greatly reduces the premature and intrapancreatic activation of digestivezymoges and attenuates the severity of experimental pancreatitis. Magnesium depletion, on the other hand, increases the susceptibility of the pancreas towards pathological stimuli. An ongoing clinical trial may document a beneficial effect of magnesium supplementation for patients at risk of recurring episodes of pancreatitis such as those with hereditary risk factors.
Prevalence of CFTR Gene Mutations in Patients with Idiopathic Chronic Pancreatitis

P. Simon¹, F.U. Weiss¹, N. Bogdanova¹, M.M. Lerch²
¹Department of Medicine B, University of Münster, ²Department of Medicine A, University of Greifswald, Greifswald, Germany

Background: Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians. Using screening panels containing the most common CFTR mutations the prevalence has been found to be increased in patients with idiopathic chronic pancreatitis. We have investigated the frequency of CFTR mutations in patients with sporadic idiopathic chronic pancreatitis by sequencing of the entire CFTR coding region.

Patients and Methods: 66 patients with idiopathic pancreatitis as determined by unequivocal CT and/or ERCP criteria were included in the study. The classification ‘idiopathic pancreatitis’ was based on exclusion of known nutritive, biliary, metabolic or endocrine risk factors of the disease. Patients with trypsinogen gene mutations as well as patients with a history or symptoms of CF were excluded. CFTR mutations were identified by sequencing of the entire coding region of the gene.

Results: A total of 5 abnormal CFTR alleles were identified in three patients (4.5%); (1 × R347P, 1 × ΔF508/D1152H, 1 × ΔF508/R117H). Neither of these patients also carried a SPINK1 gene mutation. The 5T allele was present in 8 of the 66 patients (12%) tested, but not in heterozygocity with CFTR mutations and in only one of these patients associated with a SPINK1 N34S mutation.

Conclusions: In 66 German patients with unequivocal chronic pancreatitis the allele frequency of CFTR mutations or the prevalence of a 5T allele were only marginally elevated above control levels. While this indicates greater regional variation in the association between CFTR mutations and idiopathic pancreatitis than previously expected it also indicates that compound heterozygosity may not be required for the CFTR changes to represent a risk factor for pancreatitis.

The Incidence of Hereditary Pancreatitis in Westphalia is not Related to a Founder Effect

F.U. Weiss¹, P. Simon¹, C. Hohoff², B. Brinkmann², M.M. Lerch¹
¹Department of Gastroenterology, University of Greifswald, ²Institut für Rechtsmedizin Universitätsklinikum Münster, Germany

Background: To date 17 point mutations in the cationic trypsinogen gene have been found to be associated with pancreatitis. The most frequent point mutation (R122H) in exon 3 of the trypsinogen gene is found in approx. 75% of patients with hereditary pancreatitis. Patients and families with this particular mutation have been found world-wide. However a large number of affected families originate from Westphalia in Germany. We investigated whether this is due to a potential unknown relatedness between these kindreds (‘founder effect’).

Patients and Methods: Using multiplex PCR and direct genomic sequencing we investigated non-coding mitochondrial (HV1 and HV2) polymorphisms in 34 HP patients from 16 affected families. Y-chromosomal (Y-STRs and Y-SNPs) polymorphisms were analyzed in 22 male patients.

Results: Neither mtDNA analysis nor Y-STR haplotype determination indicated a common ancestor of the investigated families. Furthermore in one kindred we could demonstrate a spontaneous R122H ‘de novo’ mutation.

Conclusions: The high prevalence of HP kindreds in Westphalia could be accounted for by either ascertainment bias in a tertiary referral center or by multiple spontaneous mutations in a trypsinogen hotspot at position R122. Our results do not support the notion of a founder effect or gene conversion as the cause of the prevalence of HP.

The Evolutionary G198R Mutation is Responsible for the Inhibitor Resistance and Substrate Restriction of Human Mesotrypsin

R. Szmola, Z. Kukor, M. Sahin-Toth
Boston University, Boston, Mass., USA

Background: Human mesotrypsin displays an unusually high resistance against naturally occurring polypeptide inhibitors. On the basis of sequence alignments [Nyaruhucha, et al: J Biol Chem 1997;272:10573] and a recent crystal structure [Katona, et al: J Mol Biol 2002;315:1209] it was suggested that the inhibitor resistance was due to a single evolutionary mutation, which replaced Gly198 with a bulky Arg residue. However, this assumption has never been tested experimentally.

Objective: Our goal was to investigate the role of the evolutionary substitution G198R in mesotrypsin function.

Methods: We performed in vitro site-directed mutagenesis on mesotrypsinogen and changed Arg198 to Gly, which is highly conserved among trypsin-like serine proteases. Properties of wild type and R198G mutant mesotrypsinogen(ogen) were compared with respect to inhibition by trypsin inhibitors,zymogen activation, and degradation.

Results: The R198G mutant formed tight-binding inhibitor complexes with human pancreatic secretory trypsin inhibitor, soy-bean trypsin inhibitor, bovine pancreatic trypsin inhibitor or eotin. As shown previously, wild-type mesotrypsin was resistant to these trypsin inhibitors. R198G-mesotrypsin also efficiently activated human cationic or anionic trypsinogen, bovine chymotrypsinogen or human pro-elastase 2. In contrast, wild-type mesotrypsin was unable to activate any of the pancreatic zymogens tested, and showed only poor activity in zymogen degradation. Finally, compared to wild-type mesotrypsin, R198G-mesotrypsin (ogen) was relatively unstable, and underwent rapid autocatalytic degradation.

Conclusions: The unique evolutionary mutation G198R appears to be solely responsible for the inhibitor resistance and
restricted substrate recognition of human mesotrypsin. In addition, the same mutation significantly stabilized mesotrypsin (ogen) against autocatalytic degradation.

Supported by NIH grant DK58088.

### 11

**Human Mesotrypsin Rapidly Degrades Trypsin Inhibitors**

Z. Kukor, R. Szmola, M. Sahin-Toth  
Boston University, Boston, Mass., USA

**Background:** Mesotrypsin is an enigmatic minor human trypsin isoform, which has been recognized for its peculiar resistance to biological trypsin-inhibitors such as human pancreatic secretory trypsin inhibitor (SPINK1) or soy-bean trypsin inhibitor (SBTI). In search of a biological function, it was proposed that the inhibitor-resistant trypsin activity could activate other pancreatic zymogens and act as a causative factor in the pathogenesis of pancreatitis. An alternative theory suggested that degradation of pancreatic zymogens might be the key function of mesotrypsin, which in this capacity would be protective rather than pathogenic. We ruled out both of these theories, and showed that the G198R evolutionary mutation not only endowed mesotrypsin with inhibitor resistance, but also significantly restricted its ability to cleave protein substrates.

**Objectives:** We hypothesized that due to their low but still significant affinity, mesotrypsin recognizes protein trypsin inhibitors as substrates and may rapidly hydrolyze their reactive-site peptide bonds. We set out to characterize the interaction of mesotrypsin with SPINK1 (model for Kazal type inhibitors) and SBTI (model for Kunitz type inhibitors).

**Methods:** Inhibition of mesotrypsin and degradation of inhibitors were followed by activity measurements, inhibition assays and gel electrophoresis.

**Results:** SBTI and SPINK1 acted as low-affinity competitive inhibitors of mesotrypsin. Mesotrypsin rapidly cleaved the reactive site peptide bond of SBTI, whereas it completely degraded SPINK1. In contrast, cationic trypsin, anionic trypsin, chymotrypsin or elastase 2 were ineffective in degrading free SPINK1.

**Conclusions:** Our results demonstrate that mesotrypsin is a unique digestive protease specialized for the degradation of trypsin inhibitors. Physiologically, mesotrypsin can facilitate digestion of foods rich in natural trypsin inhibitors. Furthermore, the findings raise the possibility that premature activation of mesotrypsinogen to mesotrypsin in the pancreas might lower protective SPINK1 levels and contribute to the development of pancreatitis.

Supported by NIH grant DK58088.

### 12

**Cathepsin B Preferentially Activates Mesotrypsinogen of the Three Human Trypsinogen Isoforms**

R. Szmola, Z. Kukor, M. Sahin-Toth  
Boston University, Boston, Mass., USA

**Background:** We demonstrated that mesotrypsin can specifically degrade human pancreatic secretory trypsin inhibitor (SPINK1), while it is defective in activating or degrading trypsinogen or other pancreatic zymogens. These observations suggested that (1) the biological function of mesotrypsin is digestive degradation of trypsin inhibitors; and (2) inappropriate activation of mesotrypsinogen inside the pancreas may reduce protective SPINK1 levels and possibly cause pancreatitis.

**Objectives:** We sought to identify the potential pathological activator(s) of mesotrypsinogen, which can liberate mesotrypsin activity in the pancreas.

**Methods:** Mesotrypsinogen was incubated with human cationic trypsin, human anionic trypsin, or the lysosomal cysteine protease, human cathepsin B. Activation and degradation of mesotrypsinogen was followed by activity assays and gel electrophoresis.

**Results:** Mesotrypsinogen did not autoactivate to any extent whatsoever. Surprisingly, cationic or anionic trypsin caused minimal activation with significant mesotrypsinogen degradation. In remarkable contrast, cathepsin B activated mesotrypsinogen potently, and compared to the two other trypsinogen isoforms, activation of mesotrypsinogen was markedly enhanced. This difference in activation was pH dependent, and it amounted to approximately 10-fold at the physiologically relevant pH 5.0 value.

**Conclusions:** Autoactivation or trypsin-mediated activation is unlikely to generate appreciable mesotrypsin activity in the pancreas. In contrast, cathepsin B strongly and preferentially activates mesotrypsinogen of the three human trypsinogen isoforms. The observation provides a biochemical basis for early intrapancreatic mesotrypsinogen activation, which might play a role in the onset of human pancreatitis.

Supported by NIH grant DK58088.

### 13

**Analysis of p53 Status in Pancreatic Juice by Yeast Functional Assay as a Potential Screening Test for Inherited Pancreatic Cancer**

Royal Liverpool University Hospital, Liverpool, Merseyside, UK

**Background:** K-ras mutations are found in up to 100% of cases of pancreatic ductal adenocarcinoma (PDAC). However, mutant K-ras...
is also found in patients with chronic pancreatitis and those with a normal pancreas. Mutations in p53 tumour suppressor gene have been reported in 23–100% of cases of PDAC and are much more specific for this disease. Data on the presence of p53 mutations in pancreatic juice in PDAC is, however, limited.

**Objectives:** Analysis of the p53 and K-ras mutation status in pancreatic juice, with a view to developing a screening test for pancreatic cancer in high risk individuals from families on the EUROPAC registry.

**Methods:** Pancreatic juice was obtained from a consecutive series of 50 patients with PDAC, 50 with chronic pancreatitis and 51 controls with gallstones. Analysis for p53 mutations utilized the yeast functional assay which has been developed to identify functional p53 mutations. Exons 5 to 8 are amplified, linked together and transformed into yeast. Mutant p53 produces red yeast colonies, from which DNA can be extracted for direct sequencing. K-ras mutations were detected with mutation-specific primers by real-time PCR.

**Results:** Mutant p53 was detected in 21/50 of cases of PDAC (sensitivity = 42%, specificity = 95%). Fifteen of the 21 patients with mutant p53 also had mutant K-ras (71%). There was one case of mutant p53 in chronic pancreatitis (2%) in a patient who also had a K-ras mutation, but had not developed pancreatic cancer at 12 months follow-up. No p53 mutations were detected in controls (20% mutant K-ras).

**Conclusions:** These results show the potential use of combined p53 and K-ras analysis of pancreatic juice sampled at ERCP as part of the screening for pancreatic cancer. This analysis in combination with other molecular analysis and detailed imaging of the pancreas may be a viable strategy for screening of individuals at risk of developing inherited pancreatic cancer.

---

**14 Angiotensin-Converting Enzyme Polymorphism is not Associated with Chronic Pancreatitis**

N. Oruc, O.C. Kutlu, M.M. Barmada, D.C. Whitcomb

University of Pittsburgh, Pittsburgh, Pa., USA

**Background:** The rennin-angiotensin system has been implied in the pathogenesis of various diseases including acute and chronic pancreatitis. An insertion (I) and deletion (D) type polymorphism in angiotensin converting enzyme (ACE) has been linked to heart disease, diabetes and even cerebrovascular disease.

**Objective:** The aim of this study was to investigate the occurrence of ACE gene polymorphism in chronic pancreatitis patients.

**Methods:** A group of 160 chronic pancreatitis patients and age-sex matched 68 healthy controls were evaluated. ACE insertion or deletion type polymorphism were determined in all subjects. All demographic and clinical data were collected by a standard personal questionnaire supplemented with information contained in the medical evaluation.

**Results:** ACE gene I and D allele frequency in chronic pancreatitis patients (50% and 50%) were similar to controls (43% and 57% respectively). The distribution of ACE genotype in chronic pancreatitis patients was II-27.5%, ID-45%, and DD-27.5%, as compared to controls with genotype of 22.1%, 41.1%, and 36.8%, respectively.

When severity of the disease and complications of chronic pancreatitis considered, there were no significant differences between patients with II, ID or DD genotypes.

**Conclusion:** ACE gene polymorphism does not contribute to pathogenesis and progression of chronic pancreatitis.

---

**15 Different Functions of E-cadherin in the Metastasis of Pancreas Tumor Cells**

J. Schnekenburger, I. Bredebusch, M.M. Lerch, W. Domschke

University of Muenster, Muenster, Germany

**Background:** E-cadherin and the associated catenin complex play a key role in epithelial cell adhesion. Tumor cells have to downregulate adhesion junction proteins for tissue infiltration whereas in distant metastases the cadherin/catenin complex is often found reexpressed. After downregulation of E-cadherin it was shown that b-catenin and p120catenin can be translocated to the nucleus and activate proliferative pathways.

**Objectives:** We analyzed the function of E-cadherin in the proliferation and invasive potential of a metastatic pancreas tumor cell line.

**Methods:** PaTu 8988T cells were retrovirally transduced with E-cadherin expression constructs or empty vectors, selected and cloned. The expression of E-cadherin and catenins was analyzed by SDS PAGE and Western Blot and immunofluorescence staining with specific antibodies. The morphology of the cell lines was examined by raster electron microscopy, proliferation by MTT growth assay and the dependence on paracrine factors by a limited dilution assay. The ability of cells to migrate was tested by an in vitro wounding assay.

**Results:** E-cadherin expressing PaTu 8988T cells showed an upregulation of b-catenin and p120catenin expression. The proliferation of these cells was slightly increased compared to mock transfected cells. E-cadherin expressing PaTu 8988T cells formed intact adherens junctions and could in contrast to mock transfected cells not escape from the cell layer in an in vitro wounding assay. E-cadherin expression increased the colony formation of PaTu 8988T cells in a limited dilution assay to the 5-fold. The morphology of E-cadherin expressing PaTu 8988T cells was strikingly different from parental or mock transfected cells. E-cadherin expressing PaTu 8988T cells lost the mostly ball-shaped epithelial phenotype and appeared as flat fibroblast-like cells growing as a monolayer.

**Conclusions:** The reexpression of E-cadherin in a metastatic pancreas carcinoma cell line alters the invasive potential of the cells not only by the formation of adherens junctions. The increased colony formation of E-cadherin expressing PaTu 8988T cells in a limited dilution assay indicates a possible role of E-cadherin in the formation of distant metastases. The morphological alterations reveal a role of E-cadherin in the organization of the cytoskeleton and suggest an additional mechanism of tumor metastasis inhibition.
16

Functional Characterization of KFL11, a Novel TGFβ-Regulated Tumor Suppressor for Pancreatic Cancer
M.E. Fernandez-Zapico, J.R. Molina, D.A. Ahlquist, R. Urrutia
Mayo Clinic, Rochester, Minn., USA

Background: Tumor suppressor genes encode proteins that antagonize the function of oncogenic pathways. One important mechanism of cancer development involves the inactivation of genes encoding these proteins by either genetic (deletions and point mutations) or epigenetic mechanisms (DNA methylation). Our laboratory studies molecular mechanisms underlying tumor suppression by the TGFβ pathway in the exocrine pancreas. Consistent with this goal, the current study focuses on the functional characterization of KLF11, a novel TGFβ-regulated tumor suppressor transcription factor that antagonizes the oncogenic function of K-Ras. Because K-Ras is mutated in more than 90% of pancreatic tumors the study of KLF11 is of critical medical relevance. This gene belongs to the KLF/Sp1-like family, a group of transcription factors involved in the modulation of different morphogenetic pathways during embryogenesis and neoplastic transformation.


Results: Colorimetric MTS assay and BrdU incorporation showed that KLF11 inhibits cell growth in pancreatic epithelial cells. Nuclear Hoechst staining and TUNEL assay demonstrated that KLF11 induces apoptosis in pancreatic cells. Foci formation assays using NIH3T3 cells demonstrated that KLF11 suppresses transformation mediated by K-Ras. Expression arrays and PCR analysis showed that KLF11 expression is decreased in pancreatic cancer (65% of the cases). Analysis of the potential mechanisms responsible of this downregulation demonstrated that the KLF11 promoter is aberrantly methylated in 50% of the pancreatic cancer cell lines analyzed and in the 72% of pancreatic tumor studied. 5-Aza-2′-deoxycytidine treatment of the KLF11 methylated pancreatic cancer cell lines led to restoration of KLF11 expression.

Conclusions: The TGFβ-regulated KLF11 protein suppresses neoplastic transformation mediated by K-Ras. The antitumoral activity of KLF11 together with its inactivation in pancreatic cancer support a role for this transcription factor as a TGFβ-regulated tumor suppressor in this tissue. Thus, together with other TGFβ-regulated tumor suppressor proteins (e.g. DPC4/Smad4), KLF11 is a novel potential drug targets in chemotherapeutic regimens aimed at reducing the oncogenic function of K-Ras in pancreatic cancer.

RU is supported by the Mayo Cancer Center, Lustgarten Foundation, for Pancreatic Cancer Research and the National Institute of Health grant DK52913 and DK56620.

17

The Interaction of a Chromosomal Form of HP1 gamma with Ku70 in Pancreatic Cancer Cells Suggest a Role for this Protein Complex in the Maintenance of Chromosomal Instability
G. Callahan, S. Delgado, D. Bensi, L. Huggins, R. Urrutia
Mayo Clinic, Rochester, Minn., USA

Abstract: Chromosomal instability is the major pathway leading to the development of pancreatic cancer. Although, the mechanisms of chromosomal instability are poorly understood, emerging evidence supports a critical role of the non-homologous end-joining (NHEJ) DNA repair pathway in this phenomenon. Ku70, a regulatory partner of DNA protein kinase, is a key mediator of this cascade. In this study, we report potential mechanisms for the regulation of NHEJ and chromosomal instability via the interaction of Ku70 with a human isoform of HP1, a protein that regulates embryogenesis in Drosophila melanogaster. We show that pancreatic cancer cells express three HP1 proteins, HP1 alpha, HP1 beta, HP1 gamma with an enrichment of the latter isoform. The pancreas-enriched isoform, HP1 gamma, is phosphorylated in tumor cells by PKA at serine 93 as determined by mutational analysis in conjunction with kinase assays. Immunofluorescence experiments using confocal microscopy and three-dimensional reconstruction demonstrate a selective association of phosphorylated HP1 gamma with chromosomes. In vitro binding assays and in vivo immunoprecipitation studies reveal that this chromosomal form of HP1 gamma specifically interacts with Ku70. Together, these results reveal the existence of a novel mechanism by which the function of Ku70 may be regulated by a chromosomal form of HP1 gamma, suggesting a role for this protein complex in the maintenance of chromosomal stability.

RU is supported by the Mayo Cancer Center, the Lustgarten Foundation and NIH Grants DK52913 and DK56620.

18

Pancreatic Cancer Collaborative Registry
S. Sherman, R. Brand, M. Ketcham, O. Shats, Y. Mashinson, D. Shats
University of Nebraska Medical Center, Omaha, Neb., USA

Abstract: Pancreatic cancer (PC) is a devastating disease with an increasing incidence in Western countries. It is the fourth-leading cause of cancer death in both men and women. The development of standardized data collection, together with a comprehensive plan for registering and collecting information on PC patients and individuals at high risk of developing PC, are fundamental needs to achieve a better knowledge of this disease. The goal of this project was to develop the Pancreatic Cancer Collaborative Registry (PCCR), a repository for socio-demographic, environmental, clinical and family history data of individuals and family members with a history of PC. The Web-page questionnaires have been designed in a format in
which personal data is obtained from and submitted by the subject; and medical data is collected and submitted by clinicians involved in the subject’s care. Collaborators from University of Nebraska Medical Center, Creighton University, John Hopkins University, University of Washington, Mayo Clinic, University of Pittsburgh, and Evanston Northwestern Healthcare have been involved in determining and reviewing the common fields of the PCCR forms. The PCCR was designed using Multi-Tier Web-based technology. All patient identifiers defined by HIPAA standards are encrypted. The PCCR is oriented on several types of end-users, each with the corresponding level of access to PC data. Initially, when a user attempts to gain access to computing resources, the user is prompted to enter his/her ID and password, which are preliminarily assigned to each user. Data collected in the PCCR will significantly expand the potential for demographic and epidemiologic studies in both familial PC, as well as seeming sporadic occurrences of this disease where the large number of registry participants and consistent data collection methods will increase validity of biostatistical analyses. We estimate that in four years there will be at least 5,000 cases entered into the PCCR from all participating sites. This will result in one of the largest outcome databases on PC patients in the world that will allow for certain multivariate and stratified analyses to be performed.

Selected Abstracts

Pancreatology 2003;3:429–441

Evidence for the Association of the 4q32-34 Locus and Pancreatic Cancer in European FPC Families


University of Liverpool, Liverpool, Merseyside, UK

Background: Familial Pancreatic Cancer (FPC) is a rare autosomal dominant disease, the causative gene of which is unknown. However, the locus 4q32-34 has been identified in one large American kindred as a potential locus for the disease. The European registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) was established at Liverpool in 1997 in order to study families with pancreatic disease. EUROPAC has so far recruited 113 families with pancreatic cancer. We also collaborate with the FaPaCa group in Germany who have additional FPC families.

Objectives: To determine whether the 4q32-34 locus is associated with pancreatic cancer in FPC families.

Methods: The 4q32-34 locus has been haplotyped in affected and unaffected individuals in 41 EUROPAC and FaPaCa families that fulfill our criteria for FPC (more than one affected first degree relative in more than one generation or more than two related individuals if not first degree). The locus was haplotyped with 9 mapping pairs between D4S413 and D4S415 from section 13 of the Genethon map of chromosome 4 covering an area of 15 cM region. The sizes of the PCR products produced with these mapping pairs are used to define an allele and haplotypes assigned to each individual.

Results: We have excluded linkage to the 4q32-34 locus in 7 of these families. Of the remaining 34 families linkage cannot be excluded due to either the lack of biological samples or that the disease status of individuals cannot be confirmed as they are considered too young to develop the disease or be classed as unaffected. A total of 55 haplotypes have been identified as possibly being linked to the disease and a further 75 haplotypes have been ruled out.

Conclusions: Linkage to the 4q32-34 locus has been excluded in 7/41 families analyzed so far. LOD score analysis will enable us to determine the likelihood of this locus being linked in all of our families. However, EUROPAC and FaPaCa continue to recruit new families in order to continue the study of this locus and to identify other potential loci.

Effect of Smoking on the Risk for Developing Pancreatic Cancer in Pancreatic Cancer Prone Kindreds

R. Brand, P. Maisonneuve, A. Lowenfels, C. Deters, H. Lynch

Evanston Northwestern Healthcare, Highland Park, Ill., USA

Background: It is estimated that 5 to 10% of pancreatic cancer cases are due to hereditary factors. The majority of these familial clusterings have an autosomal dominant pattern of inheritance, while other families develop pancreatic cancer as part of a recognized cancer syndrome. Cigarette smoking is the most significant environmental etiologic risk factor for the development of pancreatic adenocarcinoma. The aim of this study is to determine whether smoking increases the risk for pancreatic cancer in pancreatic cancer prone families enrolled in our hereditary registry.

Methods: Data on smoking was available from 260 pancreatic cancer prone families at Creighton’s Hereditary Cancer Institute. These families consisted of either 137 pancreatic cancer kindreds with two or more cases of pancreatic cancer or 123 families with a recognized hereditary syndrome known to predispose to pancreatic cancer (45 with hereditary breast cancer, 29 with familial atypical multiple mole melanoma syndrome, 31 with hereditary nonpolyposis colorectal cancer, 6 with familial adenomatous polyposis and 12 others). We restricted the analysis to pancreatic cancer cases and related family members aged over 20. Conditional logistic regression was used to assess the risk of pancreatic cancer with smoking within their families. All models were adjusted for age.

Results: We found an almost 2-fold increase risk of developing pancreatic cancer in smokers as compared to non-smokers in these pancreatic cancer prone families. This increase in pancreatic cancer was seen in both families with a recognized hereditary syndrome (odds ratio 1.74, 95% CI 1.02–2.97) as well as non-syndromic pancreatic cancer prone families (odds ratio 1.86, 95% CI 1.18–2.92). Individuals younger than 50 years also appeared to have an increased risk of developing pancreatic cancer in these high risk families (odds ratio 4.49, 95% CI 1.40–14.5).

Conclusion: These findings underscore the need for early education of these high-risk family members on smoking prevention and cessation.
Measurement of Pancreatic Cancer Risk by Mutational Load Distribution Analysis in Pancreatic Cancer-Prone Families


Yale University School of Medicine, New Haven, Conn., USA

Mutational Load Distribution Analysis (MLDA) provides the frequency distribution of mutated alleles of cancer gene(s) found in a population of somatic cells. The skewness of the distribution of the mutational load provides the major measure of risk. MLDA analysis of pancreatic juice is based on three premises: (i) mutation drift and selection are the major forces responsible for tumor formation; (ii) pancreatic juice contains soluble DNA that serves as surrogate for sampling the cells in the pancreatic exocrine system; (iii) zip arrays enable the multiplex quantification of mutated alleles for Ki-Ras (8 codon 12/13 mutations) and p53 (14 mutations in exons 5 and 7) with the capability of detecting one mutated allele in a background of 100 wild type alleles. Previous results indicated that the presence of any mutated allele below the 1.2% level classified an individual as no risk and any allele above 3.9% indicated the presence of a neoplasm. Individuals were considered at risk if levels were between 1.2% to 3.9%. The aim of this pilot study was to apply MLDA to pancreatic juice from high-risk patients to determine if this approach warranted further investigation.

Methods: Eight asymptomatic family members from 3 different p16 germline mutation kindreds underwent collection of pancreatic juice in the duodenum following secretin stimulation. Six had the p16 mutation while two were wild-type. Four members underwent serial collections (2, 2, 3, 5) over 3 years while the others had one collection. Sixteen samples were obtained and blindly analyzed by MLDA.

Results: The two wild-type members and two others were identified as no risk. Three members were found at risk on all six of their samples while a fourth member initially no risk for two samples, progressed on three subsequent samples to at risk.

Conclusions: These preliminary results indicate that MLDA of pancreatic juice, for Ki-Ras and selected exons of p53, may measure the risk of pancreatic cancer development. Further studies are planned to determine if MLDA can be an effective tool for the real time measurement of the risk for pancreatic cancer and perhaps a novel way to achieve early detection of pancreatic cancer.

Two Novel Severe Mutations in the Pancreatic Secretory Trypsin Inhibitor Gene (SPINK1) Cause Familial or/and Hereditary Pancreatitis

J.M. Chen, C. Le Maréchal, O. Raguénès, C. Férec

INSERM 0115, Génétique Moléculaire et Génétique Epidémiologique, Université de Bretagne Occidentale, Établissement Français du Sang–Bretagne, and Centre Hospitalier Universitaire de Morvan, Brest, France

Background and Aims: Mutations in the serine protease inhibitor Kazal type 1 gene (SPINK1) encoding pancreatic secretory trypsin inhibitor (PSTI) have recently been found to be associated with chronic pancreatitis. Nevertheless, knowledge of severe mutations is particularly scarce, both in terms of number and in the extent of clinical information. The aim of this study was to expand the known spectrum of such mutations.

Methods: 46 unrelated families, each including at least two pancreatitis patients and carrying neither cationic trypsinogen (PRSS1) mutations nor the frequent SPINK1 N34S mutation, participated in this study. The four exons and their flanking sequences of the SPINK1 gene were screened by denaturing high performance liquid chromatography analysis (DHPLC); and mutations were identified by direct sequencing.

Results: A heterozygous microdeletion mutation (c.27delC), which occurs within a symmetric element, was identified in two families. In one family, c.27delC showed segregation with the disease across two generations, with a penetrance of up to 75%. But in the other family, however, the same mutation manifested as a low-penetance susceptibility factor. In addition, a novel heterozygous splicing mutation, IVS2+1G>A, was found in one family with familial pancreatitis.

Conclusions: Our results demonstrated that whenever possible, mutational screening rather than genotyping should be performed, given the ‘loss-of-function’ nature of SPINK1 mutations. Moreover, genetic testing for SPINK1 mutations in pancreatitis families wherein no PRSS1 mutations were found is warranted. Furthermore, our results suggested that it might be more appropriate to assign SPINK1 as a pancreatitis susceptibility locus; and the differing views about SPINK1’s role (i.e., disease-causing vs. disease modifier) in pancreatitis should be discussed in the context of specific mutations.
33
Rapid Detection of Genomic Rearrangements in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene by Quantitative Multiplex PCR Amplification of Short Fluorescent Fragments: Extensive Allelic Heterogeneity and Diverse Mutational Mechanisms

C. Férec, J.M. Chen, O. Raguènès, M.-P. Audrézet
INSERM 0115, Génétique Moléculaire et Génétique Epidémiologique, Université de Bretagne Occidentale, Etablissement Français du Sang–Bretagne, and Centre Hospitalier Universitaire de Morvan, Brest, France

Background and Aims: Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Despite the extensive and enduring efforts of many CF researchers over the past 14 years, up to 30% of disease alleles still remain to be identified in some populations. It has long been suggested that gross genomic rearrangements could account for these unidentified alleles. To date, however, only a few large deletions have been found in the CFTR gene and only three have been fully characterized. Here we report the first systematic screening of the 27 exons of the CFTR gene for large genomic rearrangements, by means of the quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF).

Methods: A well-characterized cohort of 39 classical CF patients carrying at least one unidentified allele, after extensive and complete screening of the CFTR gene by both denaturing gradient gel electrophoresis and denaturing high-performance liquid chromatography, participated in this study.

Results: Using QMPSF, some 16% of the previously unidentified CF mutant alleles were identified and characterized, including 5 novel mutations (1 large deletion and 4 indels). The breakpoints of these 5 mutations were precisely determined enabling us to explore the underlying mechanisms of mutagenesis.

Conclusions: Although non-homologous recombination may be invoked to explain all 5 complex lesions, each mutation appears to have arisen through a different mechanism. One of the indels was highly unusual in that it involved the insertion of a short 41bp sequence with partial homology to a retrotranspositionally-competent LINE-1 element. The insertion of this ultra-short LINE-1 element may constitute a novel type of mutation causing human genetic disease.

24
Functional Isolation of Pancreatic Tumor Suppressor Genes

Y.M. Park, R.D. George, J.A. Mackey, K.L. Pogue-Geile
University of Pittsburgh Medical School, Pittsburgh, Pa., USA

Purpose: The purpose of this work is to isolate novel, functional pancreatic cancer-tumor suppressor gene(s) from two regions of the genome at 12q21.31-32 and 12q23.3 that have been shown to be frequently lost in pancreatic cancer [Kimura, et al: Cancer Research 1998;58:2456–2460].

Methods: To isolate functional tumor suppressor genes: (1) Human clones in Yeast Artificial Chromosomes (YACs) that map to 12q21.31-32 and to 12q23.3 were ‘retrofitted’ with a neomycin resistance gene (NeoR), a mammalian cell selectable marker. (2) ‘Retrofitted’ YAC clones were introduced into Panc-1, A9 and A549 cells via spheroplast fusion. Panc-1 cells were used because it is a pancreatic cancer cell line which contains an allelic loss at 12q21-23. The other cell lines were used because they have previously been shown to fuse to yeast cells. (3) NeoR colonies were isolated in some cases and are currently being evaluated for tumor suppressor activity. (4) Tumor suppressor genes will be isolated from YACs that contain tumor suppressor activity by gene expression analysis, mutation analysis and by assaying tumor suppressor activity of specific genes.

Results: We have retrofitted the NeoR gene into 5 YAC clones, 4 of which map to and completely cover the two regions of loss at 12q21.31-32 and 12q23.3 and the other YAC contains the p16 gene, which is a positive control for tumor suppressor activity. These five YAC clones have been introduced into cancer cell lines based on the appearance of NeoR colonies in at least one of 3 cell lines. The number of NeoR colonies varied widely with the YAC and cell line that was used. Parallel to this functional analysis, we have developed a targeted gene array composed of 29 clones that map to 12q21.31-32 and 12q23.3. Preliminary analysis of gene expression in this region has shown that only 3 genes show substantial down regulation in cancer, a pattern that might be expected for a tumor suppressor gene.

Conclusion: Tumor suppressor activity appears to be present in all 5 YAC clones based on the long-term survival and morphological changes that were observed. However, this activity was not present in all cell lines. Y909H1 did not limit survival of A9 cells, suggesting that tumor suppressor activity was YAC and cell line specific.

25
Identification of Markers for the Early Detection of Pancreatic Cancer

University of Pittsburgh, Pittsburgh, Pa., USA

Identification of gene expression patterns diagnostic and prognostic for specific pancreatic pathologies could aid in early diagnosis and better patient outcomes. Microarray technology provides the potential for identifying thousands of genes that are differentially expressed between cell types. We have developed the Pittsburgh Pancreas gene-Enriched Array – (PittPEAR) – which is composed of 5,763 pancreas expressed genes.

Purpose: In an effort to identify critical genes whose expression is changed early in pancreatic cancer development we have compared the gene expression pattern of normal adjacent tissue (pancreatic tissue with normal histology which is adjacent to tumor) to donor normal pancreas. Numerous studies have indicated that normal adjacent tissue is NOT normal and may contain pre-neoplastic changes.

Selected Abstracts

Pancreatology 2003;3:429–441
Results: We have identified the 50 most differentially expressed genes in two data sets. In the first data set we have identified the 50 most differentially expressed genes when 10 pancreatic adenocarcinoma samples were compared to two donor normal pancreas tissues. In the second data set we have compared 3 normal adjacent tissue to 2 donor normal pancreas tissues. We have generated a list of the most significantly differentially expressed genes from each data set. Comparisons of these two lists of differentially expressed genes are very similar. At least 50% of these genes are identical in the two lists although the rank ordering of significance varies somewhat between the two lists. Examples of genes that were among the most significantly underexpressed genes on both lists include regenerating islet-derived 1 alpha, period (Drosophila) homolog 1, pancreatitis associated protein, RNA binding motif, single stranded interacting protein and eukaryotic translation elongation factor 2. Genes that were identified as significantly overexpressed in both analyses include nucleolin, apoptosis related protein, and villin 1. By extending our comparisons to dysplastic tissue from an individual with a very high risk of developing pancreatic cancer (Family X), we will help to verify these potential early detection markers.

Conclusions: Normal adjacent tissue appears to contain many gene expression changes indicative of the neighboring cancer. We plan to exploit the phenomenon to identify an early marker for pancreatic cancer.

Potential Interest in Developing Pre-Implantation Genetic Diagnosis (PGD) for Hereditary Pancreatitis.

I.H. Ellis¹, C.D. McFaul², M.M. Lerch³, J.P. Neoptolemos²

¹Department of Clinical Genetics, Alder Hey Children’s Hospital, ²Academic Department of Surgery, Royal Liverpool University Hospital, Liverpool, UK, ³Division of Gastroenterology and Endocrinology, Ernst-Moritz-Arndt Universität Greifswald, Greifswald, Germany

Families with Hereditary Pancreatitis (HP) face numerous medical problems and uncertainty across their lifetime. In younger years patients carrying a PRSS1 mutation may experience painful attacks of recurrent acute pancreatitis, surgery and eventually pancreatic exocrine and endocrine failure. Eventually there may be up to a 40% risk of affected patients developing pancreatic cancer.

Although multi-disciplinary specialist advice and support is available from joint gastroenterology, surgical and genetics clinics, most therapy is symptomatic and supportive. Intervention studies to reduce or lessen the intensity of attacks of acute pancreatitis are still in the development phase. The EUROPAC Register of Hereditary Pancreatic Disease Families has contact with 90 families (over 270 affected individuals) in the UK and Europe that carry one of the three common PRSS1 mutations (R122H, N29I or A16V).

In the last five years only 6 families have asked to discuss reproductive options, including the possibility of pre-natal diagnosis for HP. To date none have taken up this option. Developments in pre-implantation genetic diagnosis (PGD) allow the creation of fertilised embryos in vitro, using IVF techniques. These embryos can be tested for a variety of single gene disorders, including potentially HP. We are proposing to survey families on the EUROPAC Register to assess their level of interest in PGD and potential demand for a PGD service for HP to be developed. The postal questionnaire is due to go to review by an Ethics Committee and will be presented for discussion. The critical comments of researchers and clinicians closely involved with the management of families with HP is sought to inform this process.
Author Index for Abstracts

Numbers refer to abstract number

Ahlquist, D.A. 16
Audrézet, M.-P. 23
Austin, M.A. 5, 6
Aviram, M. 2
Barmada, M.M. 14
Bartsch, D.K. 19
Bebok, S. 2
Bensi, D. 17
Bentur, L. 2
Blau, H. 2
Bogdanova, N. 8
Bowen, D.J. 5, 6
Brand, R. 18, 20, 21
Bredebusch, I. 15
Brentnall, T. 25
Brinkmann, B. 9
Burke, W. 6

Callahan, G. 17
Capella, G. 21
Chen, J.M. 22, 23
Costa, J. 21
Crispin, D. 25
Delgado, S. 17
Deters, C. 20
Domschke, W. 7, 15

Earl, J. 19
Eibl, G. 4
Ellis, I.H. 1, 3, 13, 19, 26
Evans, J. 13
Fernandez-Zapico, M.E. 16
Fesinmeyer, M.D. 5, 6
Fishbach, A. 6
Férec, C. 22, 23
George, R.D. 24, 25
Gilmore, I. 13
Greenhalf, W.G. 13, 19
Guha, S. 4
Hahn, S.A. 19
Hlouschek, V. 7
Hohoff, C. 9
Howes, N. 13
Huggins, L. 17
Kerem, B. 2
Kerem, E. 2
Kerzin-Storrar, L. 1
Ketcham, M. 18
Kisfalvi, K. 4
Korczowski, B. 3
Kress, R. 19
Kukor, Z. 10, 11, 12
Kutlu, O.C. 14
Ladner, D. 21
Le Maréchal, C. 22
Lee, K.K.W. 25
Lerch, M.M. 7–9, 15, 26
Leslie, J. 13, 19
Lizzi, P. 21
Lombard, M. 13
Lowenfels, A. 20
Lynch, H. 20, 21
Mackey, J.A. 24, 25
Maisonneuve, P. 20
Malone, K.E. 5
Mashinson, Y. 18
McFaul, C.D. 13, 19, 26
McDonald, F. 1
Molina, J.R. 16
Moore, F. 7
Moser, A.J. 25
Mountford, R. 3
Neoptolemos, J.P. 1, 3, 13, 19, 26
Oruc, N. 14
Park, Y.M. 24
Pogue-Geile, K.L. 24, 25
Potter, J.D. 5, 6
Raguénès, O. 22, 23
Rieder, H. 19
Rivlin, J. 2
Rozengurt, E. 4
Sahin-Toth, M. 10–12
Schnekenburger, J. 7, 15
Shats, D. 18
Shats, O. 18
Sherman, S. 18
Shushan, L. 2
Simon, P. 8, 9
Sina-Frey, M. 19
Smart, H. 13
Sutton, R. 13
Szmola, R. 10–12
Taraffa, G. 21
Threadgold, J. 13
Topazian, M. 21
Turi, S. 7
Urrutia, R. 16, 17
Weiss, F.U. 8, 9
Whitcomb, D.C. 14, 25
Wilschanski, M. 2
Wong, T. 13
Wood, P.G. 25
Yaakov, Y. 2
Yahav, J. 2
Yan, L. 13, 19