15. Obara T, Maguchi H, Saitoh Y, et al. Mucin-producing tumor of the pancreas: a unique clinical entity. Am J Gastroenterol 1991; 86:1619–1625.
16. Obara T, Saitoh Y, Maguchi H, et al. Papillary adenoma of the pancreas with excessive mucin secretion. Pancreas 1992; 7:114–117.
17. Yamaguchi K, Tanaka M. Mucin-hypersecreting tumor of the pancreas with mucin extrusion through an enlarged papilla. Am J Gastroenterol 1991; 86:835–839.
18. Bos JL. Ras oncogenes in human cancer: a review. Cancer Res 1989; 49:4682–4689.
19. Almoguera C, Shibata D, Forrester K, et al. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 1988; 53:549–554.
20. Friess H, Berberat P, Schilling M, et al. Pancreatic cancer: the potential clinical relevance of alterations in growth factors and their receptors. J Mol Med 1996; 74:35–42.
21. Smit VTHB, Boot AJM, Smits AMM, et al. K-ras codon 12 mutations occur very frequently in pancreatic adenocarcinoma. Nucleic Acids Res 1988; 16:7773–7782.
22. Barbacid M. ras Genes. Ann Rev Biochem 1987; 56:779–827.
23. Santos E, Nebreda AR. Structural and functional properties of ras proteins. FASEB J 1989; 3:2151–2163.
24. Shibata D, Capella G, Perucho M. Mutational activation of the c-K-ras gene in human pancreatic carcinoma. Baillieres Clin Gastroenterol 1990; 4:151–169.
25. Neuman WL, Wasylyshyn ML, Jacoby R, et al. Evidence for common molecular pathogenesis in colorectal, gastric, and pancreatic cancer. Genes Chromosomes Cancer 1991; 3:468–473.
26. Nagata Y, Abe M, Motoshima K, et al. Frequent glycine-to-aspartic acid mutations at codon 12 of c-Ki-ras gene in human pancreatic cancer in Japanese. Jpn J Cancer Res 1990; 81:135–140.
27. Motojima M, Tsunoda T, Kanematsu T, et al. Distinguishing pancreatic carcinoma from other periampullary carcinomas by analysis of mutations in the Kirsten-ras oncogene. Ann Surg 1991; 214:657–662.
28. Mariyama M, Kishi K, Nakamura K, et al. Frequency and types of point mutation at the 12th codon of the c-Ki-ras gene found in pancreatic cancers from Japanese patients. Jpn J Cancer Res 1989; 80:622–626.
29. Lemoine NR, Jain S, Hughes CM, et al. Ki-ras oncogene activation in preinvasive pancreatic cancer. Gastroenterology 1992; 102:230–236.
30. Gruenwald K, Lyons J, Froehlich A, et al. High frequency of Ki-ras codon 12 mutations in pancreatic adenocarcinomas. Int J Cancer 1989; 43:1037–1041.
31. Gonzalez–Cadavid NF, Zhou D, Battifora H, et al. Direct sequencing analysis of exon 1 of the c-K-ras gene shows a low frequency of mutations in human pancreatic adenocarcinomas. Oncogene 1989; 4:1137–1140.
32. Hruban RH, van Mansfeld ADM, Offerhaus GJA, et al. K-ras oncogene activation in adenocarcinoma of the human pancreas. Am J Pathol 1993; 143:545–554.
33. Burmer GC, Rabinovitch PS, Loeb LA. Frequency and spectrum of c-Ki-ras mutations in human sporadic carcinoma, carcinomas arising in ulcerative colitis and pancreatic adenocarcinoma. Environ Health Perspect 1991; 93:27–31.
34. Capella G, Cronauer–Mitra S, Pienado MA, Perucho M. Frequency and spectrum of mutations at codons 12 and 13 of the c-K-ras gene in human tumors. Environ Health Perspect 1991; 93:125–131.
35. Satoh K, Shimosegawa T, Moriizumi S, et al. K-ras mutation and p53 protein accumulation in intraductal mucin-hypersecreting neoplasms of the pancreas. Pancreas 1996; 12:362–368.
36. Satoh K, Sawai T, Shimosegawa T, et al. The point mutation of c-Ki-ras at codon 12 in carcinoma of the pancreatic head region and in intraductal mucin-hypersecreting neoplasm of the pancreas. Int J Pancreatol 1993; 14:135–143.
37. Sessa F, Solcia E, Capella C, et al. Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-ras, p53 and c-erbB-2 abnormalities in 26 patients. Virchows Arch 1994; 425:357–367.
38. Tada M, Omata M, Ohto M. Ras gene mutations in intraductal papillary neoplasms of the pancreas. Analysis in five cases. Cancer 1991; 67:634–637.
39. Yanagisawa A, Kato Y, Ohtake K, et al. c-Ki-ras point mutation in ductectatic-type mucinous cystic neoplasm of the pancreas. Jpn J Cancer Res 1991; 82:1057–1060.
40. Rall CJ, Yan YX, Graeme–Cook F, et al. Ki-ras and p53 mutations in pancreatic ductal adenocarcinoma. Pancreas 1996; 12:10–17.
41. Light R, Margolin BH. Analysis of variance for categorical data. J Am Stat Assoc 1971; 66:534–544.
42. Rivera JA, Rall CJN, Graeme–Cook F, et al. Analysis of K-ras oncogene mutations in chronic pancreatitis with ductal hyperplasia. Surgery 1997; 121:42–49.
43. Terada T, Ohta T, Nakanuma Y. Expression of oncogene products, anti-oncogene products and oncofetal antigens in intraductal papillary-mucinous neoplasm of the pancreas. Histopathology 1996; 29:355–361.
44. Hoshi T, Imai M, Ogawa K. Frequent K-ras mutations and absence of p53 mutations in mucin producing tumors of the pancreas. J Surg Oncol 1994; 55:84–91.

Discussion

DR. JOHN L. CAMERON (Baltimore, Maryland): I enjoyed this paper nicely presented by Dr. Z'graggen, and I think it represents the type of work that we are going to be seeing presented at this Association more and more in the future, and less and less on how to manage the various pancreatic tumors surgically.

As we heard earlier in the meeting, there are good operations for pancreatic tumors that can be performed on virtually all patients in every age group, and I don't think we are going to see much more in the way of progress in the operative management of these tumors.

The problem is early detection. At least two thirds of our patients with pancreatic adenocarcinoma have metastatic tumor in regional nodes at the time of resection. What we need is a means of detecting these tumors before they have spread to regional nodes.

Six or eight years ago there was only one solid tumor about which anything was known concerning the molecular events that preceded the development of malignancy. That was from work done by Dr. Bert Vogelstein at Johns Hopkins demonstrating so beautifully the multiple molecular events that accompany the changes from normal colonic mucosa, to polyp formation, to high-grade dysplasia, carcinoma *in situ,* and then in invasive cancer. At that time, 6 or 8 years ago, there was virtually nothing known about the molecular events that accompany the transformation of ductal epithelium in the pancreas to malignancy.

But that has changed. In the course of just 6 or 8 years

pancreatic cancer has gone from a tumor in which virtually nothing was known to one that is one of the better studied of the solid tumors.

I think it is interesting to point out that the work done at Johns Hopkins, Massachusetts General, and other institutions around the world was stimulated by the increased interest in this tumor clinically.

There was virtually no one working at Johns Hopkins on the molecular events that precede the development of pancreatic cancer before clinical material became plentiful. And I dare say at Massachusetts General, until Dr. Warshaw started attracting a large number of patients with pancreatic cancer, that there was very little molecular work being done on this disease.

Point mutations of codon 12 of the K-*ras* oncogene, the focus of this presentation by Dr. Z'graggen, is a mutation that is seen in association with many of the prevalent solid tumors—breast, colon, and lung, to name just three—but in none of those is it present in more than, say, 50% of patients. In a series at Johns Hopkins that now approaches 200 patients who have been well characterized, this point mutation is present in more than 90%.

As the paper presented today points out, it is probably a very early mutation in the development of pancreatic cancer, which I think gives hope that it might be of some benefit in screening patients. It has been demonstrated at Johns Hopkins that one can actually detect this mutation in the stool of patients with pancreatic cancer. Part of our SPORE grant on pancreatic cancer supports an attempt to develop a stool screening test for this point mutation in large populations of patients. Obviously, if this mutation were picked up in a stool specimen it would not be specific for pancreatic cancer, but it could put the spotlight on a group of patients who should then be screened with a computed tomographic scan, tumor markers, even perhaps endoscopic retrograde cholangiopancreatography.

In closing, I would like again to congratulate Dr. Z'graggen, Dr. Warshaw, and their group at Massachusetts General for a nice study and would like to ask them whether they have started, either with the mutation at codon 12 of the K-*ras* oncogene or any of the other molecular markers that are being described virtually daily, to work on a screening test, perhaps combining duodenal secretions, stool, blood, or other samples that might result in selecting a high-risk group for pancreatic cancer?

DR. MURRAY F. BRENNAN (New York, New York): The authors have considerable experience with these very unusual tumors and have made significant contributions to their clinical and biologic understanding. As you saw, they examined 16 patients with intraductal papillary mucin-producing tumors for *ras* mutations and found a high prevalence of a codon 12 mutation.

The object in looking for these mutations was an effort, as Dr. Cameron suggested, not just to understand the genetic events but actually to use it for both diagnosis and possibly even treatment.

We examined 96 patients with ampullary tumors thinking that perhaps we would be able to discriminate ampullary origin from pancreatic adenocarcinoma. We found, as the authors have in this study, that the K-*ras* mutation occurred early, appearing in adenomas, in carcinoma *in situ,* and in frank carcinoma. In contradistinction to the 90% seen by the Johns Hopkins group in pancreatic adenocarcinoma and the approximately 80% here (13 of 16), in ampullary tumors the prevalence was 30% to 40%, more like that seen in colon carcinoma.

The findings today would suggest that these tumors are of ductal origin. Although the authors showed some stepwise increase in frequency with progressive malignancy, I might question their conclusions. There was no difference in the manuscript, which I have had a chance to review, between normal duct and hyperplasia and low-grade dysplasia, or between low-grade dysplasia and carcinoma. Although the trend was different, the difference between 16% and 30% when the denominator is 16 is the difference between 3 of 16 and 5 of 16.

Dr. Warshaw, Dr. Z'graggen, I might question the conclusion in the manuscript that quotes, "It is an important event in transformation from normal epithelium to invasive carcinoma," and would more likely suggest that it is an early event in neoplastic transformation rather than an important event in discriminating between benignity and malignancy.

Nevertheless, these observations can only add to our knowledge about these difficult tumors. I would also ask if they might speculate on how they might use such strategies both in diagnosis and in treatment.

DR. L. WILLIAM TRAVERSO (Seattle, Washington): I would like to also congratulate the Massachusetts General Hospital group for having the foresight of doing the K-*ras* investigations in this fairly large series of patients.

Now, I am not going to quibble with the statistics, but their data suggests that a point K-*ras* mutation may be an early event in degenerating to malignancy in the pancreatic duct system. K-*ras* gene mutations also appear more frequently during the transformation from dysplasia into malignancy. These are the important contributions in this study.

I would like to focus on the clinical side for those who treat these patients and are looking for ways to help make treatment decisions. As you heard Dr. Cameron say, there are basically three ways that we can improve the treatment of our patients with pancreatic malignancy. One is to extend the margins until they are negative. Two is to design better chemoradiotherapy or adjuvant protocols. Probably the most promising one is the third one, early diagnosis.

I would like to call your attention to the University of Washington's gastroenterology laboratory and Dr. Teresa Brentnall. We have been sending our pancreatic juice obtained during endoscopic retrograde cholangiopancreatography (ERCP) to her laboratory. In mucinous duct-ectatic neoplasms we have resected, we find in those with benign-type histology that the K-*ras* mutations will not be found in the pancreatic juice. If they tend toward the malignant side then K-*ras* mutations will be found in pancreatic juice.

After presentation of this Massachusetts General study the finding is really not surprising, because the epithelium of the pancreatic duct turns over very rapidly. DNA material appears in the pancreatic juice and, if collected, can provide some information. Do you, Dr. Warshaw, find that pancreatic juice may be a good way to perform an early diagnostic maneuver? In regard to the diffuse nature of the disease, however, you saw

that it occurred within various areas within the tumor. My problem with these patients is about half of them will present with hyperamylasemia. The computed tomographic scan will show a small cyst, but the ERCP shows a duct ectatic mucin-secreting neoplasm. The endoscopist can see mucus streaming out of the ampulla of Vater.

These patients may have more of a benign form of disease. But if there is a stricture involved, then malignancy is more likely.

In your 15 patients, the disease is diffuse in about a third of the cases. In our series of 31 cases it is diffuse in more than half of the cases. What do you do then in these older patients? We have taken out the tail, we have taken out the head, and in a number of these cases they have recurred with symptoms even though it is benign. Symptoms were primarily those of recurrent pancreatitis. Could you give us some guidelines on the extent of resection?

DR. KEITH A. KELLY (Scottsdale, Arizona): My question is a little off the mark.

How often are ductile papillary changes found in what appears to be "garden-variety" adenocarcinoma of the pancreas? When papillary changes are found, what should the surgeon do? Should he resect to the point of normal duct, which could mean an extensive pancreatectomy? Or should these changes be followed in some other way?

DR. MICHAEL T. LOTZE (Pittsburgh, Pennsylvania): Vogelstein has indeed informed us about the pathway of multistep carcinogenesis. One of the questions I would ask is whether you have examined other genetic events, p16 or p53 mutations, which occur frequently in these and other neoplasms, and whether you might distinguish the earliest events, which are presumably *ras* mutations, and some of the perhaps later genetic events associated with neoplastic transformation. In addition, to follow up on Dr. Cameron's suggestion about earlier diagnosis, Whitcomb and Ehrlich in Pittsburgh recently identified a syndrome associated with mutations in the trypsin gene associated with hereditary pancreatitis and a marked increase in the frequency of subsequent carcinoma developing in the pancreas. The question is whether there are any of the hereditary syndromes in which *ras* mutations have been examined as perhaps an early marker for progression to pancreatic cancer.

DR. KASPER Z'GRAGGEN (Closing Discussion): With regard to the statistics, we analyzed the distribution of the populations of data rather than comparing each subgroup against the others. That analysis shows that there is a statistically significant difference in the prevalence of K-*ras* mutation across the subgroups.

Dr. Kelly asks about hyperplasia commonly found in association with ductal adenocarcinoma. Our focus of study, however, was on the papillary lesions—both hyperplasia and neoplasia—that are part of the spectrum found in intraductal papillary mucinous tumors of the pancreas. This should be viewed differently from the hyperplasia associated with ductal adenocarcinoma.

The important question to us is, what are the clinical implications of these findings? The K-*ras* mutations found not only in the neoplasm but also in nearby seemingly normal ductal epithelium suggest that this represents a field defect with the potential of subsequent development of further neoplasm in the pancreatic remnant. Should we recommend routine total pancreatectomy in these patients as a consequence? We do not think so at present inasmuch as we have seen only one instance of a second tumor developing in the pancreatic remnant in 20 years of experience.

In our recent report of 25 patients with intraductal papillary mucinous tumors we have chosen to resect the affected segment when the disease was limited to either the left or, more commonly, the right side. However, when the entire duct is involved or if the frozen sections of the ducts at the transection margins repeatedly show papillary growth we proceed to a total pancreatectomy. Among the 25 cases, 6 clearly involved the entire duct and clear margins could not be achieved.

Just a short comment regarding screening. Endoscopic retrograde cholangiopancreatography (ERCP) is an invasive technique and cannot be considered a screening tool. K-*ras* also is unlikely to be useful for screening both because the mutation does not occur universally and because it can occur at any stage of development of the tumor. We do not have experience with screening stool samples for the K-*ras* mutation. It is fair to say that a true screening test for any form of pancreatic neoplasm has yet to be developed.