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Fanconi Anemia Gene Mutations in Young-onset Pancreatic Cancer[1]

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ABSTRACT

Genes of the Fanconi complementation groups [Fanconi anemia (FA) genes] are suggested to be involved in homologous DNA recombination and produce FA when two allelic mutations are inherited. *BRCA2* is an FA gene and additionally conveys an inherited risk for breast, ovarian, and pancreatic cancer for individuals carrying a single mutated allele [N. G. Howlett *et al.*, Science (Wash. DC), *297:* 606–609, 2002]. Here we report inherited and somatic mutations of *FANCC* and *FANCG* present in young-onset pancreatic cancer. This may imply a general involvement of Fanconi genes with an inherited risk of cancer. The known hypersensitivity of Fanconi cells to mitomycin and other therapeutic agents [M. S. Sasaki, Nature (Lond.), *257:* 501–503, 1975] suggests a therapeutic utility for a more complete characterization of the DNA repair defects and their causative genetic mutations in pancreatic cancer.

INTRODUCTION

FA[3] is an inherited autosomal recessive syndrome, characterized cellularly by hypersensitivity to cross-linking agents such as mitomycin C. Patients often present with congenital bone deformities and bone marrow failure and are highly susceptible to the occurrence of hematological tumors (especially acute myelogenous leukemia) and squamous cell tumors of the head and neck, gynecological system, and other organs. Recently, *BRCA2* mutations have been shown to be responsible for a subset of FA patients, complementation group D1. Cells from these patients were recently reported to be complex heterozygous, containing both hypomorphic and nonfunctional alleles of *BRCA2*; complementation of such cells with wild-type *BRCA2* cDNA restored resistance to mitomycin C (1, 2). Among human tumors, pancreatic cancers harbor the highest prevalence of *BRCA2* mutations, which are present in 7–10% of "sporadic" pancreatic cancers (those with a family history negative for cancer) and 17% of families with a strong history of the disease [kindred with ≥3 family members affected, including at least 2 first-degree relatives (3–6)]. As is *BRCA2*, the *FANCC* and *FANCG* genes are the sites of additional FA gene mutations carried in the general population. To help understand the potential inherited basis of pancreatic cancer, we determined whether inactivating *FANCC* and *FANCG* gene mutations might occur as homozygous mutations in pancreatic cancers.

MATERIALS AND METHODS

Cancers of the pancreas and distal common bile duct resected at the Johns Hopkins Hospital between 1992 and 1997 were expanded as xenografts in immunodeficient mice as described previously (7). At the time of surgery, resected normal duodenum was frozen and stored at −80°C. *FANCC* was sequenced using automated capillary sequencing of PCR products in 22 tumors selected for LOH at 9q22.3[4] and 11 unselected pancreatic cancer cell lines (BxPC3, AsPC1, CAPAN1, CAPAN2, MiaPaCa2, Panc-1, Hs766T, CFPAC1, Su86.86, PL9, and PL45) obtained from the American Type Culture Collection (Manassas, VA) and European Collection of Animal Cell Cultures (Salisbury, United Kingdom). *FANCG* was sequenced in 22 tumors selected for LOH at 9p13 and the same 11 cell lines used for *FANCC*.

All exons were amplified and sequenced from genomic DNA. Mutations were confirmed by sequencing of independent PCR products and confirmed in case PX19 by analysis of two xenografts derived independently from the same primary tumor. Constitutional DNA, where available, was sequenced to determine whether the alterations were somatic or germ-line in origin.

FANCC exons were amplified using the following PCR primers: (*a*) 5′-AGAGCCTTTTAGAAATGCTTC and 5′-CCTGAAGTCAGAAAATAATTTC, exon 1; (*b*) 5′-CCCATTTAAGGATGAAGT and 5′-CATACATGGACAACAGTATAG, exon 2; (*c*) 5′-ATGTTATATTCAGGGATACTTG and 5′-TAACAGTGAAGGGTATGTTTG, exon 3; (*d*) 5′-TAGGTAAAGCACTGCTCATTG and 5′-TGGCACATTCAGCATTAAAC, exon 4; (*e*) 5′-ACAGAGTGAAACATGAGAAG and 5′-AACATCCATTTCCTATGAATT, exon 5; (*f*) 5′-TGTTCATCGATGGTGTTAGAG and 5′-TGTCGTACAGTCTTTCCAA, exon 6; (*g*) 5′-GATGAGAAGTCTCACAAATTG and 5′-ATTATATATAAAGGTTCCAATTG, exon 7; (*h*) 5′-AGGAGTATACAGAGGAATAAG and 5′-ACTCTAATTTCCCCATGATAC, exon 8; (*i*) 5′-TCACACAAGGACTGAAATCTG and 5′-AAGTGCTCTTGTCCAAAATAC, exon 9; (*j*) 5′-TGTTCTGACCATGTTAGTAC and 5′-AATGCTCTTCCCAGGAAATC, exon 10; (*k*) 5′-TCCGTGAACCAGAAGTAAAG and 5′-TGGTCCCAGACCAGTAATG, exon 11; and (*l*) 5′-CAGTGGATAAGTACAATTTAAG and 5′-GCAGGTTGCCATGACATATG, exon 12. The following PCR primers were used for *FANCG*: (*a*) 5′-CTCGGCGGGGTGCAGAA and 5′-CCCGAGTAATTATATCGATC, exons 1 and 2; (*b*) 5′-GGGTGGGTTCTTTATTGTAG and 5′-AGACAACTAGCACTCAACTAG, exons 3 and 4; (*c*) 5′-GGTCTAGCCAGGATAGATG and 5′-AGTGCTCTCTGTGGATTTC, exons 5 and 6; (*d*) 5′-GGGAAACCACAAGCATTATG and 5′-GAGGAGTGGCGACCTATG, exons 7 and 8; (*e*) 5′-ATCCATACTGAGCCAAAATTG and 5′-CAGTCTTGCTGTATTTCAAAG, exon 9; (*f*) 5′-TGGTGTCCCCTTTGAAATAC and 5′-AGGGTAAGTAGGTGAACATG, exon 10; (*g*) 5′-GGTGTGGAGGGATGATTTTC and 5′-AACACCACTCTTACACTTAC, exons 11 and 12; and (*h*) 5′-GCCTAAGACTATGTCAAGTTC and 5′-AGACAACTAGCACTCAACTAG, exons 13 and 14.

RESULTS AND DISCUSSION

Disease-inducing intragenic *BRCA2* mutations in pancreatic cancer are uniformly accompanied by loss of the wild-type allele (4); we therefore studied conventional ("sporadic") ductal pancreatic adenocarcinomas that had LOH at 9q22.3 (*FANCC*) and 9p13 (*FANCG*). Three homozygous variants were observed: one in *FANCG*; and two in *FANCC* (Table 1; Fig. 1). Specifically, a deletion of 5 bp (nucleotides 1903–1907; National Center for Biotechnology Information) was observed in exon 14 of *FANCC*, resulting in a frameshift alteration. Upon comparison with the nonneoplastic DNA of this patient, this mutation was found to be somatic. The other *FANCC* mutation was a germ-line missense mutation in exon 6: 839 A>T, D195V. This mutation has been described previously in a FA patient and was associated with a milder clinical presentation (8). The *FANCG* mutation of the Hs766T cell line is identical to a mutation common in FA patients with a German ancestry and leads (in its homozygous form)

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[3] The abbreviations used are: FA, Fanconi anemia; LOH, loss of heterozygosity.

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Table 1 *Fanconi gene mutations in pancreatic cancer*[a]

Gene and setting	Mutations	LOH[b]	Origin	Age (yrs)
BRCA2 (familial) (reference population)	17%	−	Inherited	66.0 66.7
BRCA2 (sporadic) (reference population)	7–10%	LOH	Usually inherited	71.5 64.3
FANCC (sporadic)				
PX19 tumor	D195V (GAT to GTT)	LOH	Inherited	44
PX102 tumor	Frameshift (CCTTAAA to CA)	LOH	Somatic	47
CAPAN2 cell line	E521K (GAG to AAG)[c]	No LOH	Unknown	56
FANCG (sporadic)				
Hs766T cell line	E105ter (GAG to TAG)	LOH	Probably inherited	46

[a] *BRCA2* data are from Refs. 4–6. Average ages for *BRCA2* mutations include only inherited mutations. PX19 and PX102 had no family history of cancer by chart review. Variants present in the constitutional DNA were considered inherited.
[b] LOH in tumors resulting in homozygosity for the mutation.
[c] Functional significance unknown. −, not studied.

to a relatively early onset of the disorder (9, 10). In addition to these homozygous mutations, one heterozygous mutation was observed in *FANCC* exon 14, 1813G>A, E521K in cell line CAPAN2. This allele was confirmed to be expressed upon analysis of cDNA. No mutation was found in the other allele. The functional significance has not yet been determined; the mutation is not reported in FA patients or as a normal polymorphism.

These findings echo prior studies of *BRCA2* suggesting that low-penetrance mutant alleles, ones that are not uncommon among the unsuspecting general population, might also serve as a widespread

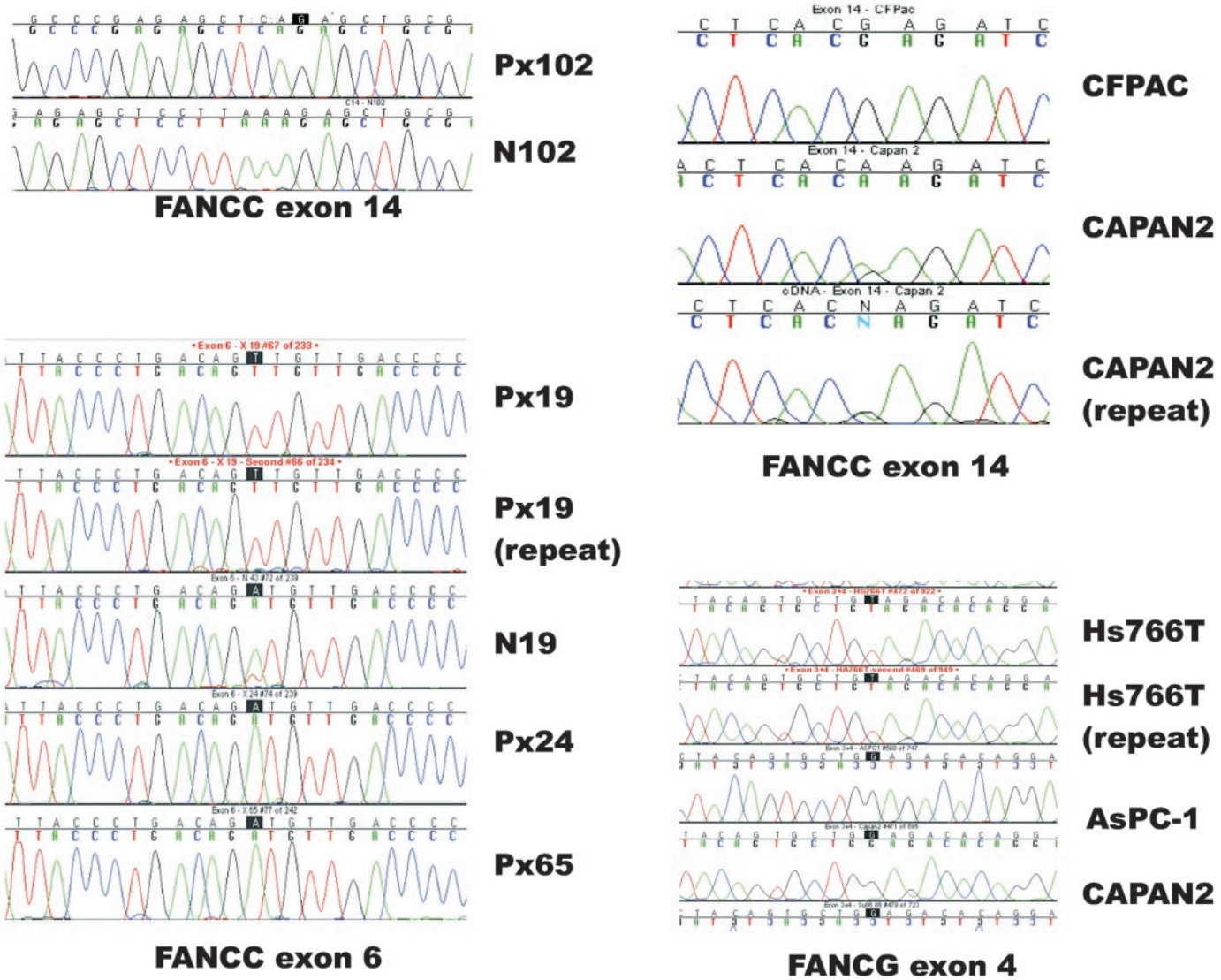

Fig. 1. Chromatograms of mutations identified in pancreatic cancers. All sequences depicted are from genomic DNA except for the CAPAN2 repeat, which represents cDNA sequence.

cause of an inherited risk of cancer among a population. "Sporadic" cancer is not always sporadic, an inconvenient but instructive terminological problem (4). The *BRCA2* example is now extended by the tumor-associated mutations of *FANCC* and *FANCG* genes. The carrier rate of FA gene mutations has not been directly assessed but is estimated at 1 in 300 individuals in the general population (11), with higher rates in identifiable subpopulations.

Unlike *BRCA2* mutations, which may not convey a decreased age of onset of pancreatic cancer (4, 6), the inherited FA gene mutations of Hs766T and tumor PX19 involved patients that are unusually young for pancreatic cancer (Table 1). Interestingly, the *FANCC* homozygous mutation in PX102, although of somatic origin, also affected a relatively young patient. The mutations here affected a third of the patients less than 50 years of age (three of nine patients) in our study population (samples of the remaining four young patients that lacked LOH were subsequently sequenced; no mutations were found). Both *FANCC*-mutant tumors were from nonsmokers. All four cases with sequence variants were otherwise histologically and genetically typical; they were ductal adenocarcinomas with aneuploidy and multiple genetic abnormalities known from prior studies (12). In the *CDKN2A*, *KRAS2*, *TP53*, and *MADH4* genes, for example, the following changes are seen, respectively: in PX19, homozygous deletion, mutation, mutation, and homozygous deletion; in PX102, homozygous deletion, mutation, mutation, and mutation; in CAPAN2, mutation, mutation, wild-type, and wild-type; and in Hs766T, mutation, mutation, wild-type, and homozygous deletion.

FA proteins appear to be ubiquitously expressed among proliferating normal cells in culture. Fancc (558 amino acids; M_r 63,000) and Fancg (622 amino acids; M_r 68,000) proteins assemble together with Fanca, Fance, and Fancf proteins in a nuclear complex, which is believed to mediate the monoubiquitination of Fancd2 protein in response to DNA damage. Subsequently, Fancd2 is targeted to nuclear foci, the formation of which may involve Brca1 protein (13). *BRCA1*$-/-$ and *BRCA2*$-/-$ cells share mitomycin C hypersensitivity with all FA cells, and wild-type BRCA2 restores resistance in *BRCA2*$-/-$ cells (1). The precise role of BRCA2 in the FA pathway has not yet been elucidated, but its BRC repeats are likely to serve as a scaffold for assembly of the RAD51 filament (14, 15). Mouse gene knockout models and the human disease of FA, however, indicate a potential difference between BRCA2 and other FA genes. Whereas the homozygous null state for most FA genes is not incompatible with live birth and viability, BRCA2-deficient cells appear to require retention of at least one conditional or hypomorphic allele to retain viability (16).

Breaks produced during the repair of mitomycin interstrand cross-links may accumulate in the absence of an intact homologous repair system in cells deficient in members of the FA complex. Recently, Hs766T has indeed been found to be especially sensitive to mitomycin C as measured by p53 responses in a high-throughput compound screening system (17). Similar compound screening may identify other therapeutics specific for cells with such defects. It has been suggested that *BRCA2*-null tumors may offer an especially wide therapeutic window for the use of chemotherapeutic agents that require homologous recombination for their repair (3, 18, 19). Using therapies that included mitomycin, occasional complete remissions of pancreatic cancer have been reported (20–24), although the *BRCA2* and FA gene status of such occasional patients has not been reported.

A wide spectrum of hematological and nonhematological cancers has been reported in FA patients. However, FA patients have apparently not been observed to have an increased rate of pancreatic cancer (25, 26). Although other nonhematological malignancies are reported in such patients, most FA patients may not survive to an age highly susceptible to pancreatic cancer. It also remains possible that due to random variance in observations, the association could be missed in any general characterization of the Fanconi population but could be more easily seen in a focused study of pancreatic cancers. The relationship of pancreatic cancer to *BRCA2* mutations, for example, is best seen in studies of pancreatic cancer families (6, 27). As another example, the high rate of pancreatic cancer in Peutz-Jeghers syndrome largely escaped notice until recently (28).

Additional study of FA genes is needed to determine possible mutations in other FA genes in pancreatic cancer or mutations in these genes among other tumor types. The stage at which FA mutations play a role in the initiation and progression of pancreatic cancer warrants investigation. Future clinical and preclinical studies should attempt to identify through genetic testing and optimize the therapeutic dosing of the subgroup of patients whose tumors contain FA defects (3).

Note Added in Proof

We recently identified additional homozygous variants in cell lines Su86.86 (*FANCC*.M350V.ATG to GTG, 57 y.o.) and CAPAN1 (*FANCG*, S7F, TCT to TTT. 40 y.o.). Neither variant is reported in Fanconi anemia patients.

REFERENCES

1. Howlett, N. G., Taniguchi, T., Olson, S., Cox, B., Waisfisz, Q., De Die-Smulders, C., Persky, N., Grompe, M., Joenje, H., Pals, G., Ikeda, H., Fox, E. A., and D'Andrea, A. D. Biallelic inactivation of BRCA2 in Fanconi anemia. Science (Wash. DC), *297:* 606–609, 2002.
2. Sasaki, M. S. Is Fanconi's anaemia defective in a process essential to the repair of DNA cross links? Nature (Lond.), *257:* 501–503, 1975.
3. Kern, S. E., Hruban, R. H., Hidalgo, M., and Yeo, C. J. An introduction to pancreatic carcinoma genetics, pathology, and therapy. Cancer Biol. Ther., *1:* 607–613, 2002.
4. Goggins, M., Schutte, M., Lu, J., Moskaluk, C. A., Weinstein, C. L., Petersen, G. M., Yeo, C. J., Jackson, C. E., Lynch, H. T., Hruban, R. H., and Kern, S. E. Germ-line *BRCA2* gene mutations in patients with apparently sporadic pancreatic carcinomas. Cancer Res., *56:* 5360–5364, 1996.
5. Ozcelik, H., Schmocker, B., Di Nicola, N., Shi, X. H., Langer, B., Moore, M., Taylor, B. R., Narod, S. A., Darlington, G., Andrulis, I. L., Gallinger, S., and Redston, M. Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. Nat. Genet., *16:* 17–18, 1997.
6. Murphy, K. M., Brune, K. A., Griffin, C., Sollenberger, J. E., Petersen, G. M., Bansal, R., Hruban, R. H., and Kern, S. E. Evaluation of candidate genes *MAP2K4, MADH4, ACVR1B,* and *BRCA2* in familial pancreatic cancer: deleterious *BRCA2* mutations in 17%. Cancer Res., *62:* 3789–3793, 2002.
7. Caldas, C., Hahn, S. A., da Costa, L. T., Redston, M. S., Schutte, M., Seymour, A. B., Weinstein, C. L., Hruban, R. H., Yeo, C. J., and Kern, S. E. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat. Genet., *8:* 27–31, 1994.
8. Verlander, P. C., Lin, J. D., Udono, M. U., Zhang, Q., Gibson, R. A., Mathew, C. G., and Auerbach, A. D. Mutation analysis of the Fanconi anemia gene FACC. Am. J. Hum. Genet., *54:* 595–601, 1994.
9. de Winter, J. P., Waisfisz, Q., Rooimans, M. A., van Berkel, C. G., Bosnoyan-Collins, L., Alon, N., Carreau, M., Bender, O., Demuth, I., Schindler, D., Pronk, J. C., Arwert, F., Hoehn, H., Digweed, M., Buchwald, M., and Joenje, H. The Fanconi anaemia group G gene FANCG is identical with XRCC9. Nat. Genet., *20:* 281–283, 1998.
10. Demuth, I., Wlodarski, M., Tipping, A. J., Morgan, N. V., de Winter, J. P., Thiel, M., Grasl, S., Schindler, D., D'Andrea, A. D., Altay, C., Kayserili, H., Zatterale, A., Kunze, J., Ebell, W., Mathew, C. G., Joenje, H., Sperling, K., and Digweed, M. Spectrum of mutations in the Fanconi anaemia group G gene, FANCG/XRCC9. Eur. J. Hum. Genet., *8:* 861–868, 2000.
11. Schroeder, T. M., Tilgen, D., Kruger, J., and Vogel, F. Formal genetics of Fanconi's anemia. Hum. Genet. *32:* 257–288, 1976.
12. Rozenblum, E., Schutte, M., Goggins, M., Hahn, S. A., Lu, J., Panzer, S., Zahurak, M., Goodman, S. N., Hruban, R. H., Yeo, C. J., and Kern, S. E. Tumor-suppressive pathways in pancreatic carcinoma. Cancer Res., *57:* 1731–1734, 1997.
13. Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M. S., Timmers, C., Hejna, J., Grompe, M., and D'Andrea, A. D. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol. Cell, *7:* 249–262, 2001.
14. Pellegrini, L., Yu, D. S., Lo, T., Anand, S., Lee, M., Blundell, T. L., and Venkitaraman, A. R. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. Nature (Lond.), *420:* 287–293, 2002.
15. Davies, A. A., Masson, J. Y., McIlwraith, M. J., Stasiak, A. Z., Stasiak, A., Venkitaraman, A. R., and West, S. C. Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. Mol. Cell, *7:* 273–282, 2001.
16. D'Andrea, A. D., and Grompe, M. The Fanconi anaemia/BRCA pathway. Nat. Rev. Cancer, *3:* 23–34, 2003.
17. Sohn, T. A., Bansal, R., Su, G. H., Murphy, K. M., and Kern, S. E. High-throughput measurement of the Tp53 response to anticancer drugs and random compounds using

a stably integrated Tp53-responsive luciferase reporter. Carcinogenesis (Lond.), *23:* 949–957, 2002.
18. Moynahan, M. E., Cui, T. Y., and Jasin, M. Homology-directed DNA repair, mitomycin-C resistance, and chromosome stability is restored with correction of a Brca1 mutation. Cancer Res., *61:* 4842–4850, 2001.
19. Tutt, A., Bertwistle, D., Valentine, J., Gabriel, A., Swift, S., Ross, G., Griffin, C., Thacker, J., and Ashworth, A. Mutation in Brca2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. EMBO J., *20:* 4704–4716, 2001.
20. Todd, K. E., Gloor, B., Lane, J. S., Isacoff, W. H., and Reber, H. A. Resection of locally advanced pancreatic cancer after downstaging with continuous-infusion 5-fluorouracil, mitomycin-C, leucovorin, and dipyridamole. J. Gastrointest. Surg., *2:* 159–166, 1998.
21. Takada, T., Nimura, Y., Katoh, H., Nagakawa, T., Nakayama, T., Matsushiro, T., Amano, H., and Wada, K. Prospective randomized trial of 5-fluorouracil, doxorubicin, and mitomycin C for non-resectable pancreatic and biliary carcinoma: multicenter randomized trial. Hepatogastroenterology, *45:* 2020–2026, 1998.
22. Sadoff, L., and Latino, F. Complete clinical remission in a patient with advanced pancreatic cancer using mitomycin C-based chemotherapy: the role of adjunctive heparin. Am. J. Clin. Oncol., *22:* 187–190, 1999.
23. Miura, T., Endo, Y., Matumoto, Y., and Ikeda, H. Intra-arterial infusion chemotherapy in combination with microwave hyperthermia for cancer of head of pancreas and liver metastasis: a case of 16 years survival. Gan To Kagaku Ryoho, *27:* 1794–1800, 2000.
24. Vaughn, C., Chapman, J., Chinn, B., Ward, D., Groshko, G., Maniscalco, B., Reznik, S., and Piper, D. Activity of 5-fluorouracil, mitomycin C, and methyl CCNU in inoperable adenocarcinoma of pancreas. Am. J. Clin. Oncol., *12:* 49–52, 1989.
25. Kutler, D. I., Singh, B., Satagopan, J., Batish, S. D., Berwick, M., Giampietro, P. F., Hanenberg, H., and Auerbach, A. D. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood, *101:* 1249–1256, 2003.
26. Alter, B. P. Cancer in Fanconi anemia, 1927–2001. Cancer, *97:* 425–440, 2003.
27. Hahn, S. A., Greenhalf, B., Ellis, I., Sina-Frey, M., Rieder, H., Korte, B., Gerdes, B., Kress, R., Ziegler, A., Raeburn, J. A., Campra, D., Grutzmann, R., Rehder, H., Rothmund, M., Schmiegel, W., Neoptolemos, J. P., and Bartsch, D. K. BRCA2 germline mutations in familial pancreatic carcinoma. J. Natl. Cancer Inst. (Bethesda), *95:* 214–221, 2003.
28. Giardiello, F. M., Brensinger, J. D., Tersmette, A. C., Goodman, S. N., Petersen, G. M., Booker, S. V., Cruz-Correa, M., and Offerhaus, J. A. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology, *119:* 1447–1453, 2000.

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