The Fanconi anemia/BRCA2 pathway in pancreatic cancer
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CHAPTER 8

THE GENETICS OF FANCC AND FANCG IN FAMILIAL PANCREATIC CANCER

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Abstract
Patients with Fanconi Anemia (FA) display a wide variety of defects including bone marrow failure and a high risk of developing cancer. Multiple FA genes exist whose proteins form a complex that along with BRCA1 is important for the translocalization of FANCD2 to nuclear foci. With BRCA2 and RAD51, this complex is thought to have a role in the repair of DNA double strand breaks. Recently, Howlett et al. identified biallelic inactivating mutations of the BRCA2 gene as the genetic basis of FA complementation group D1. Since carriers of germline BRCA2 mutations have an increased risk of developing pancreatic cancer, the FA pathway has been analyzed for inactivating mutations by van der Heijden et al., who identified FANCC and FANCG mutations in patients with pancreatic cancer. Here, we determined the role of germline FA gene mutations in kindreds in which several family members had pancreatic cancer. Sequence analysis of 38 individuals with familial pancreatic cancer enrolled in the National Familial Pancreatic Tumor Registry (NFPTR) revealed previously identified polymorphisms within two exons and one intron of FANCC, and in three introns of FANCG. In addition, an unaffected relative from one family contained an exonic polymorphism within the FANCC gene. These and published data suggest the possibility that although germline and somatic mutations in FANCC and FANCG may contribute to the occurrence of pancreatic cancers, the pancreatic cancers that arise apparently do so in a sporadic fashion rather than with a phenotype of familial pancreatic cancer. FANCC and FANCG mutations may have low penetrance for the pancreatic cancer phenotype.

Introduction
Patients with Fanconi anemia (FA) display a wide variety of defects including congenital abnormalities, bone marrow failure, and an increased susceptibility to cancer especially acute myeloid leukemia (AML) and head and neck cancer. The diagnosis of FA is based on identifying an acute sensitivity of lymphocyte chromosomes to DNA crosslinking agents such as mitomycin C (MMC) and diepoxybutane. Prior to the identification of FA susceptibility genes, complementation studies identified eight FA complementation groups that are now known to correspond to genes that function in the repair of double stranded DNA breaks and DNA-interstrand crosslinks. Seven FA genes (FANCA, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG) have been cloned.
Proteins encoded by five of these complementation groups (A, C, E, F, and G) form a complex that is important in the activation of FANCD2. FANCD2 activation leads to the formation of nuclear foci with BRCA1. Recently, Howlett et al. found that patients with FA arising from complementation groups B and D1 have germline biallelic mutations of the BRCA2 gene. Carriers of one defective germline BRCA2 mutation have an increased risk of developing breast, ovarian, and pancreatic cancer. Indeed, germline BRCA2 mutations can be found in approximately 4-7% of apparently sporadic pancreatic cancer patients. Further, among patients with familial pancreatic cancer (those in which at least two first-degree relatives have pancreatic cancer), germline BRCA2 mutations are present in approximately 17% of patients. These findings raised the possibility that genetic alterations of other members of the FA pathway could also contribute to inherited pancreatic cancer. Recently, van der Heijden et al. identified FANCC and FANCG gene mutations in pancreatic cancer. In this study, we examined patients with familial pancreatic cancer for FANCC and FANCG germline alterations in order to determine the role of FA mutations in families with pancreatic cancer. Sequence analysis identified polymorphisms within the introns and exons of the FANCC and FANCG genes. Another exonic polymorphism was found in FANCC from a family member without pancreatic cancer.

Material and Methods
Germline DNA was obtained from 58 pancreatic cancer patients enrolled in the National Familial Pancreatic Tumor Registry (NFPTR). Affected patients from kindreds with 2 or more family members with a history of pancreatic cancer were selected for this study. All kindreds and samples were assigned a coded number to assume patient confidentiality. Our Institute Review Board approved the study. Pancreatic cancer patients are listed according to coded sample numbers preceded by the letter P. P1 family members not diagnosed with pancreatic cancer are listed by a coded number preceded by F. An exception is P56 who has pancreatic cancer and is the daughter of P1. The sequences of all 14 exons of the FANCG gene as well as the sequences of 10 of 14 exons of the FANCC gene were analyzed. FANCC exons 2, 5, 7, and 10 were not analyzed because no known germline mutations were identified in these exons. Exon 1 of FANCC was also sequenced from seven members (of one kindred) not diagnosed with PC to determine the
inheritance of the 77C>T polymorphism. Primers used for the PCR amplification of FANCC and FANCG have been described. PCR products were sequenced at the Johns Hopkins University School of Medicine Biosynthesis and Sequencing Facility with an ABI 3700 automated capillary sequencer. Sequences were analyzed with Sequencher (Gene Codes Corp.). Sequence variants were confirmed by sequencing independent PCR products.

Results
Genomic DNA from 38 patients with familial pancreatic cancer was examined for possible disease causing mutations in FANCC and FANCG. Ten exons of FANCC and all 14 exons of FANCG were analyzed by sequencing. The intronic regions immediately flanking these exons were

Figure 8.1 Electropherogram of the FANCC polymorphisms. Coded sample numbers are indicated. Affected bases are boxed. Sequences matching wildtype are listed below sequences with polymorphisms. A 77C>T B 28T>G C IVS8+80G>A.
Figure 8.2 Segregation of cancer and FANCC 77C>T within a pancreatic cancer family. This is a family having members with pancreatic cancer. Those with pancreatic cancer (red), lymphoma (yellow), or breast cancer (blue) are indicated. A coded number preceded by F list P1 family members not diagnosed with pancreatic cancer. An exception is P36 who has pancreatic cancer and is the daughter of P1. Figures with dots represent individuals carrying FANCC 77C>T. Coded sample numbers are listed below representations of members whose FANCC exon1 were sequenced. All other relatives were not sequenced at this locus. (*) indicates the FANCC 28T>G variation found in one family member (F4).

also sequenced. Sequencing revealed polymorphisms in 1 exon (77C>T) and 1 intron of FANCC and in 3 introns of FANCG in patients with pancreatic cancer. Analyzing the frequency of 77C>T in other family members from the kindred with the 77C>T variant revealed an additional polymorphism (28T>G), in a family member without cancer. Exon 1 of FANCC from P1 contains the 77C>T variant (Figure 8.1A), leading to the amino acid change S26F. The P1 pedigree (Figure 8.2) shows the frequency of the 77C>T polymorphism and identifies members with cancer. Family members with pancreatic cancer (red), lymphoma (yellow), or breast cancer (blue), do not show cosegregation with 77C>T. Exon 1 of FANCC also contained a sequence variation in F4 at 28T>G, with a deduced amino acid change of C10G (Figure 8.1B). An intronic
polymorphism. IVS8+80 G>A was located in three pancreatic cancer patients (Figure S.1C).

Several intronic polymorphisms were identified in FANCG: IVS1+77A/C, IVS3+126T/C, and IVS5+58 C/T. These intronic polymorphisms are not thought to be functionally significant because they are not within splice sites or known regulatory regions. In addition, these variants are prevalent in the normal population and therefore are unlikely to be disease causing.

Discussion

In this study, we find in our population that germline FANCC and FANCG gene mutations do not contribute to the clustering of pancreatic cancers seen in the setting of familial pancreatic cancer. Several known FANCC and FANCG polymorphisms were found as well as novel polymorphisms, but based on prior studies and this study, none of these polymorphisms are thought to be functionally significant. One 77C>T FANCC polymorphism results in a nonconservative amino acid change of a serine to a phenylalanine. This nucleotide polymorphism has been previously documented\textsuperscript{151}. The lack of segregation of this variant with disease in the affected kindred suggests that 77C>T is not disease causing.

Our results suggest that mutations in the FANCC and FANCG genes are probably not a common cause of inherited pancreatic cancer where multiple family members have pancreatic cancer. However, this finding does not rule out the possibility that other FA gene alterations may contribute to familial PC. FANCA alterations are the most frequently FA causing mutations accounting for approximately two thirds of all cases of FA, while FANCC and FANCG mutations each only account for 12\% of FA cases\textsuperscript{171}. With a FA carrier rate in the United States population estimated between 1 in 200 to 1 in 500, it is possible that a low prevalence of FANCC and FANCG mutations would be evident within a larger population of familial pancreatic cancer patients\textsuperscript{172}. At this time, estimates as to the cancer risk of FA carriers are largely unknown.

These results are in contrast to the pancreatic cancer risk with germline BRCA2 mutation carriage, where the average risk of developing pancreatic cancer in carriers is approximately 5\%, and mutations in BRCA2 are the commonest known cause of familial pancreatic cancer. One potentially important clinical implication of finding germline FA
gene mutations is that loss of FA pathway function in affected patients may render their cancers hypersensitive to DNA damaging agents such as MMC or DEB\textsuperscript{165, 162}.

Despite some advances in our understanding of familial pancreatic cancer\textsuperscript{52, 59, 44, 144, 173, 174} we still do not understand the genetic basis of most forms of familial pancreatic cancer. It will be important to examine other genes in the FA pathway to determine if the loss of function of these genes contributes to pancreatic cancer development. For example, inactivation of \textit{FANCD2} in mice not only leads to a Fanconi anemia phenotype, but also leads to the development of epithelial cancers\textsuperscript{175}. In addition, Jin \textit{et al.}\textsuperscript{176} recently demonstrated that menin, the protein encoded by \textit{MEN1}, interacts with Fancd2 and that cells lacking menin are sensitive to DNA damage. Mutations in the \textit{MEN1} gene give rise to the multiple endocrine neoplasia type 1 syndrome, a syndrome characterized by pancreatic islet cell tumors and other endocrine tumors, but a distinct lack of common epithelial cancers such as pancreatic ductal adenocarcinoma.