

ONLINE MUTATION REPORT

Genetics of the *FANCA* gene in familial pancreatic cancer

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Fanconi anaemia (FA) is a rare autosomal recessive disease that is characterised by bone marrow failure, pancytopenia, and an increased susceptibility to cancers. Recently, D'Andrea and coworkers identified biallelic *BRCA2* gene mutations as a cause of FA.¹ Because of the role of *BRCA2* gene mutations in pancreatic cancer development, their findings suggested other members of the FA pathway may be targeted for genetic inactivation in pancreatic cancer. Indeed, somatic and inherited mutations of *FANCC* and somatic mutations in *FANCG* were subsequently identified in patients with apparently sporadic pancreatic cancer.² These data led to analysis of the *FANCC* and *FANCG* genes in the germline of families with multiple pancreatic cancers, but no mutations were identified.³ In most populations, *FANCA* is the most commonly mutated gene in patients with FA.⁴⁻¹⁴ In this study we determined if *FANCA* gene mutations predispose to the development of familial pancreatic cancer.

METHODS

Subjects

Lymphocyte DNA was analysed from patients with familial pancreatic cancer enrolled in the National Familial Pancreatic Tumor Registry.¹⁵ Patients with pancreatic cancer were selected if they had at least two or more first degree relatives with pancreatic cancer (mean (SD) age of 66.7 (12.3) years, males 50.3%). Variants were analysed in 110 additional patients with familial pancreatic cancer. To determine the carrier frequency of c.2574C>G (p.Ser868Arg), we analysed three control populations: healthy spouses of patients with familial pancreatic cancer (115 samples from spouses with a mean (SD) age of 66.9 (11.3) years, males 43.1%), patients who had undergone cholecystectomy (65 samples matched in age with sporadic cases) for non-malignant disease at Johns Hopkins Hospital, and individuals undergoing routine screening colonoscopy (668 samples) at the Mayo Clinic. The mean age of the colonoscopy controls was similar to our pancreatic cancer population (mean (SD) age of 59.3 (12.3) years, males 52.9%).

Lymphocyte DNA was obtained from spousal controls and colonoscopy controls, and formalin fixed paraffin embedded DNA from the resected gallbladders of hospital controls. None of the controls had a prior history of pancreatic cancer or colorectal cancer. We also analysed germline DNA from 124 individuals with apparently sporadic pancreatic cancer (those without a known family history of pancreatic cancer; mean (SD) age of 66.23 (10.3) years, males 48%). This study was approved by the Johns Hopkins Joint Committee for Clinical Investigation and the Mayo Clinic IRB.

FANCA analysis

FANCA (Genbank # NM_000135.1) was analysed using a combination of heteroduplex analysis and DNA sequencing. A detailed description of methods used including primer sequences can be accessed at <http://www.pathology2.jhu.edu/pancreas/FANCA> and at <http://jmg.bmjournals.com/supplemental/>. The Fanconi Anemia Mutation Database

Key points

- *FANCA* was examined as a candidate susceptibility gene for familial pancreatic cancer by using heteroduplex analysis and sequencing of lymphocyte DNA from 44 patients with familial pancreatic cancer.
- Several exonic variants were identified including two novel, c.377C>G (p.Thr126Arg) and c.661A>G (p.Met221Val), and one disease associated variant, c.2574C>G (p.Ser858Arg).
- The prevalence of the c.2574C>G variant in additional familial pancreatic cancer cases was similar to that of control individuals without cancer.
- Despite finding a disease associated variant in multiple individuals with familial pancreatic cancer, our results suggest that germline *FANCA* gene mutations do not contribute to familial pancreatic cancer susceptibility.

at <http://www.rockefeller.edu/fanconi/mutate/jumpa.html> to identify previously documented variants and their frequencies. Nucleotide position +1 corresponds to the A of the ATG translation initiation codon 1 (methionine).

RESULTS AND DISCUSSION

A total of 43 exons were examined in 44 DNA samples by heteroduplex analysis (1892 PCR products). A total of 375/1892 PCR products had heteroduplex alterations and sequence alterations were found in 342 of the 375 exons with PCR heteroduplexes. The 392 alleles tested shared 48 different *FANCA* sequence variations. These included 12 exonic variants (table 1) and 36 intronic variants (table 2). Of the 48 variants found, one is an intronic insertion, another is an intronic deletion, and the rest were single base pair changes, the majority of which were transitions.

The FA associated variant c.2574C>G (S858R), first documented by Wijker *et al*,¹⁶ was identified in two patients (P1 and P2) with familial pancreatic cancer. The primary cancer from these patients was not available, but germline DNA was available from only one family member from the same kindred as P1 who was also diagnosed with pancreatic cancer. This individual, the father of P1, was diagnosed at age 80 and developed pancreatic cancer much later than his son (age 49). The father of P1 did not harbour the c.2574C>G variant.

In an additional 110 patients with familial pancreatic cancer, three contained c.2574C>G. In all, five of 154 familial samples contained c.2574C>G (odds ratio (OR) 1.530, 95% confidence interval (CI) 0.560 to 4.181). The c.2574C>G variant was also seen in one of 124 patients with sporadic pancreatic cancer (OR 0.380, 95% CI 0.050 to 2.87), in 0 of 65

Abbreviations: FA, Fanconi anaemia

Table 1 Exonic *FANCA* sequence variants in pancreatic cancer

Exon	Nucleotide change	Familial†	Sporadic‡	Controls	Amino acid change	FA database§
4	c.377C>G	1/44(G)	ND	0/115	p.Thr126Arg	N
7	c.661A>G	1/44(G)	ND	0/115	p.Met221Val	N
13	c.1143G>T	6/44(T)	ND	ND	None	Y
14	c.1235C>T	3/44(T)	ND	ND	p.Ala412Val	Y
16	c.1501G>A	12/44(A)	ND	ND	p.Gly501Ser	Y
22	c.1927C>G	8/44(G)	ND	ND	p.Pro643Ala	Y
26	c.2426A>G	19/44(G)	ND	ND	p.Glu809Asp	Y
27	c.2574C>G*	5/154(G)	1/124	18/848	p.Ser858Arg	Y
30	c.2901C>T	5/44(T)	ND	ND	None	Y
33	c.3263C>T	7/44(T)	ND	8/115	p.Ser1088Phe	Y
37	c.3654A>G	7/44(G)	ND	ND	None	Y
38	c.3807G>C	5/44(C)	ND	ND	None	Y

*FA associated variant; †frequency is shown as the number of alleles containing the nucleotide in parentheses over the total number of alleles examined from familial pancreatic cancer samples; ‡ND, not determined; §Y indicates present and N indicates not present in the Fanconi Anemia Mutation Database at <http://www.rockefeller.edu/fanconi/mutate/jumpra.html>. DNA numbering is based on the cDNA sequence. The GenBank reference sequence and version number NM_000135.1 was used. Position +1 corresponds to the A of the ATG translation initiation codon. Protein sequences are numbered with the initiator methionine as codon 1.

cholecystectomy controls, in three of 115 spousal controls, and in 15 of 668 colonoscopy controls (table 1). We also determined that there was no loss of heterozygosity at the *FANCA* locus of a pancreatic cancer xenograft generated from a familial pancreatic cancer patient with a c.2574C>G variant. The evidence that the c.2754C>G (p.Ser858Arg)

variant is likely to be disease causing is based on reports in the literature that it has been found in four unrelated patients with FA who, apart from inactivation of their second allele by mutation, had no other genetic explanation for their FA.¹⁷ In addition, *FANCA* protein harbouring the p.Ser858Arg amino acid change is unable to monoubiquitinate *FANCD2*.¹⁸ Thus, the *FANCA* p.Ser858Arg variant appears to cause FA in individuals whose other germline allele is also mutated and produces a protein lacking normal *FANCA* function. However, this variant is not conserved in mice, and since we did not find this variant more often in individuals with familial pancreatic cancer than in controls, our genetic epidemiological evidence indicates that the *FANCA* p.Ser858Arg variant does not contribute to familial pancreatic cancer.

The c.3263C>T variant in exon 33, which was previously considered a mutation,¹⁶ was recently shown to be a polymorphism.¹⁹ Our results also confirm this (OR 1.19, 95% CI 0.85 to 7.45) (table 1). Electropherograms corresponding to heteroduplex analysis of exons 27 and 33 are shown in fig 1. Two novel variants of uncertain significance were identified, c.377C>G (p.Thr126Arg) and c.661A>G (p.Met221Val), which were not found in 115 spousal controls. These variants are not conserved in mouse or rat (codon 126 is valine and codon 221 is an isoleucine).

One obstacle to determining the significance of variants is the paucity of surgically resected cancers from affected carriers of suspicious variants to demonstrate the presence or absence of biallelic inactivation of *FANCA*. Another problem is the lack of segregation data for these variants, which is the result of obstacles inherent to studying the genetics of familial pancreatic cancer. Since pancreatic cancer usually occurs in older individuals and usually displays only moderate penetrance within a family, it is difficult to track the inheritance of suspected disease causing sequence alterations with sufficient numbers of affected individuals in a family to infer disease causality. For example, of ~1400 kindred enrolled in our National Familial Pancreas Tumor Registry (as of August 2004), only 188 (~14%) contain three or more individuals with pancreatic cancer. Furthermore, since the interval between pancreatic cancers in these families can span decades and since pancreatic cancer is usually rapidly fatal, generally DNA is obtainable from only a small proportion of affected individuals.

Cells that lack normal *FANCA* function are hypersensitive to DNA damage and individuals with FA have a greatly increased risk of multiple cancers.²⁰ Since *FANCA* is a DNA repair gene, cells from carriers of heterozygous mutations that underwent somatic inactivation of the remaining wild-type allele would be expected to have an increase in mutation

Table 2 Intronic *FANCA* sequence variants in familial pancreatic cancer

Intron	Nucleotide change	Frequency*	FA database†
3	c.283+44T>C	2/44(C)	N
3	c.284-103T>C	1/44(C)	N
3	c.284-151C>T	1/44(T)	N
4	c.426+68A>G	8/44(G)	N
7	c.710-12A>G	24/44(A)	Y
8	c.792+52C>G	1/44(G)	N
8	c.792+81_82del	1/44	N
10	c.894-30A>G	7/44(G)	N
12	c.1084-93C>T	18/44(T)	N
12	c.1084-49G>C	19/44(C)	Y
12	c.1084-29A>G	12/44(G)	Y
13	c.1226-20A>G	14/44(G)	Y
18	c.1715+82T>C	16/44(C)	Y
19	c.1777-29T>C	7/44(C)	N
20	c.1826+15T>C	9/44(C)	Y
20	c.1826+30insGT	7/44	Y
22	c.2014+42G>T	8/44(T)	Y
22	c.2015-71G>A	2/44(A)	N
25	c.2316+67A>G	2/44(G)	N
25	c.2316+96G>T	1/44(T)	N
27	c.2602-36G>T	1/44(T)	Y
27	c.2602-46T>A	1/44(A)	N
28	c.2778+55G>T	1/44(T)	N
28	c.2779-7T>C	5/44(C)	N
31	c.3066+55A>G	15/44(G)	N
31	c.3067-4T>C	6/44(C)	Y
31	c.3067-23G>A	6/44(A)	Y
31	c.3067-57A>C	6/44(C)	Y
32	c.3240-42G>A	12/44(A)	Y
34	c.3408+45G>A	6/44(A)	Y
35	c.3513+62C>T	8/44(T)	Y
37	c.3765+37A>G	6/44(G)	N
38	c.3829-82C>G	6/44(G)	Y
39	c.3935-16C>T	6/44(T)	Y
39	c.3935-102C>T	1/44(T)	N
42	c.4260+29C>T	24/44(C)	Y

*Frequency is shown as the number of alleles containing the nucleotide in parentheses over the total number of alleles examined from familial pancreatic cancer samples; †Y indicates present and N indicates not present in the Fanconi Anemia Mutation Database at <http://www.rockefeller.edu/fanconi/mutate/jumpra.html>. DNA numbering is based on the cDNA sequence. The GenBank reference sequence and version number NM_000135.1 was used. Position +1 corresponds to the A of the ATG translation initiation codon. Protein sequences are numbered with the initiator methionine as codon 1.

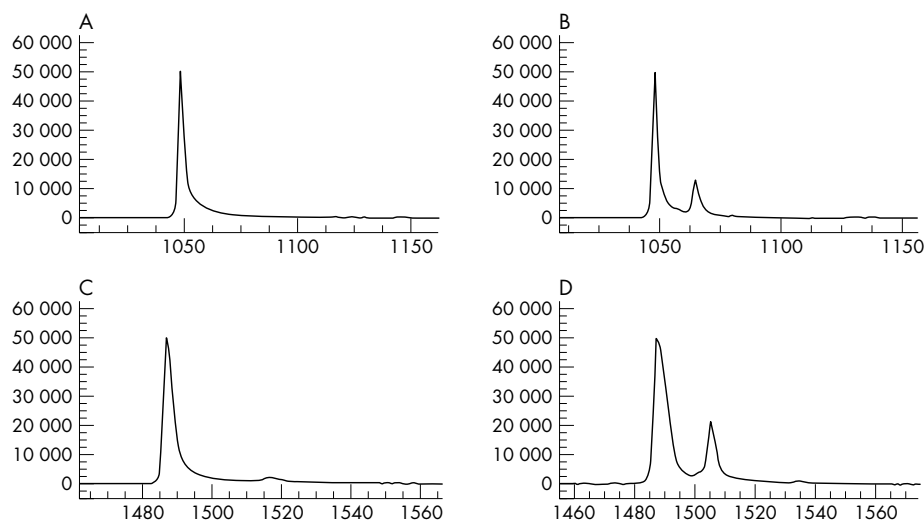


Figure 1 Heteroduplex analysis of the *FANCA* c.2574C>G and c.3263 C>T variants. DNA fragments containing exons 27 and 33 were subjected to temperature gradient capillary gel electrophoresis. Electropherograms corresponding to heteroduplex analysis of exons 27 and 33 are shown. Panels A and B show exon 27 control and c.2574C>G variant results, respectively. Panels C and D show exon 33 control and c.3263 C>T variant results, respectively. The x axis represents the migration of the amplicon and the y axis represents the relative fluorescence units.

rate and increased likelihood of cancer development. However, it has been difficult to demonstrate that heterozygote carriers of most *FANCA* gene mutations have an increased risk of developing cancer,^{21, 22} with the exception of *BRCA2*. Although pancreatic cancer is not one of the cancers that commonly occur in patients with FA, this could arise from the greater risk of developing other cancers. In addition, the occurrence of cancers in affected individuals may also be highly dependent on environmental exposures.²³ However, the results of this study, our previous study of *FANCC* and *FANCG* in familial pancreatic cancer, and the lack of germline mutations in Fanconi genes in familial breast cancers families¹⁹ suggest that any contribution of heterozygote germline *FANCA* gene mutations to cancer predisposition is likely to be a modest one.¹⁹

Recent work (Kern and coworkers) confirms that pancreatic cancer cells with inactivation of Fanconi genes are hypersensitive to mitomycin C.^{24, 25} Given the lack of useful chemotherapeutics for pancreatic cancer, it is imperative that methods are developed to identify cancers with inactivation of the Fanconi pathway. This sensitivity to mitomycin C may also extend to cancers with inactivation of *BRCA2* (*FANCD1*) as well as to those with *FANCF* inactivation due to epigenetic silencing by DNA methylation.^{26–28}

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ELECTRONIC-DATABASE INFORMATION



A detailed description of methods used can be accessed at <http://www.pathology2.jhu.edu/pancreas/FANCA> and at <http://img.bmjournals.com/supplemental/>. The Fanconi Anemia Mutation Database can be found at <http://www.rockefeller.edu/fanconi/mutate/jumpa.html>.

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Conflict of interest: none declared.

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