Genetics of the FANCA gene in familial pancreatic cancer

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METHODS

Subjects

Lymphocyte DNA was analysed from patients with familial pancreatic cancer enrolled in the National Familial Pancreatic Tumor Registry. 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 control individuals without cancer.

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.bmjjournals.com/supplemental/. The Fanconi Anemia Mutation Database online mutation report

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 (pThr126Arg) and c.661A>G (p.Met221Val), and one disease associated variant, c.2574C>G (p.Ser868Arg).
- 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.

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 sequence alterations (1892 PCR products). A total of 375/392 with FANCA gene mutations were subsequently identified in 115 samples from spouses with a mean (SD) age of 66.7 (12.3) years, males 50.3%, and 36 intronic variants (table 2). Of the 48 variants found, one is an intronic deletion, 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 (p.Ser868Arg), first documented by Wijker et al., 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
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 in 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.Ser585Arg) 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. In addition, FANCA protein harbouring the p.Ser585Arg amino acid change is unable to monoubiquitinate FANCD2. Thus, the FANCA p.Ser585Arg 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.Ser585Arg variant does not contribute to familial pancreatic cancer.

The c.3263C>T variant in exon 33, which was previously considered a mutation, was recently shown to be a polymorphism. 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. 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

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**Table 1** Exonic FANCA variant sequences in familial pancreatic cancer

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Familial†</th>
<th>Sporadics‡</th>
<th>Controls</th>
<th>Amino acid change</th>
<th>FA database†</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>c.377C&gt;G</td>
<td>1/44(G)</td>
<td>ND</td>
<td>0/115</td>
<td>p.Thr126Arg</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>c.661A&gt;G</td>
<td>1/44(G)</td>
<td>ND</td>
<td>0/115</td>
<td>p.Met221Val</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>c.1143G&gt;T</td>
<td>6/44(T)</td>
<td>ND</td>
<td>None</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>c.1235C&gt;T</td>
<td>3/44(T)</td>
<td>ND</td>
<td>None</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>c.1501G&gt;A</td>
<td>12/44(A)</td>
<td>ND</td>
<td>p.Gly412Val</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>c.1927G&gt;T</td>
<td>8/44(G)</td>
<td>ND</td>
<td>None</td>
<td>p.Pro643Ala</td>
<td>Y</td>
</tr>
<tr>
<td>26</td>
<td>c.2426A&gt;G</td>
<td>19/44(G)</td>
<td>ND</td>
<td>None</td>
<td>p.Glu809Asp</td>
<td>Y</td>
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<tr>
<td>27</td>
<td>c.2547C&gt;G*</td>
<td>5/154(G)</td>
<td>1/124</td>
<td>18/848</td>
<td>p.Ser585Arg</td>
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<tr>
<td>30</td>
<td>c.2901C&gt;T</td>
<td>5/44(T)</td>
<td>ND</td>
<td>None</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>c.3262C&gt;T</td>
<td>7/44(T)</td>
<td>ND</td>
<td>None</td>
<td>Y</td>
<td></td>
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<tr>
<td>37</td>
<td>c.3654A&gt;G</td>
<td>7/44(G)</td>
<td>ND</td>
<td>None</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>c.3807G&gt;C</td>
<td>5/44(C)</td>
<td>ND</td>
<td>None</td>
<td>Y</td>
<td></td>
</tr>
</tbody>
</table>

*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/jumpa.html. DNA numbering is based on the cDNA sequence. The GenBank reference sequence and version number NW_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.

**Table 2** Intronic FANCA variant sequences in familial pancreatic cancer

<table>
<thead>
<tr>
<th>Intron</th>
<th>Nucleotide change</th>
<th>Frequency*</th>
<th>FA database†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>c.283+4AT&gt;C</td>
<td>2/44(C)</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>c.284–103T&gt;C</td>
<td>1/44(T)</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>c.284–151C&gt;T</td>
<td>1/44(T)</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>c.426–68A&gt;G</td>
<td>8/44(G)</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>c.710–12A&gt;G</td>
<td>24/44(A)</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>c.792–52C&gt;G</td>
<td>1/44(G)</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>c.792–81_82del</td>
<td>1/44</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>c.894–30A&gt;G</td>
<td>7/44(G)</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>c.1084–93C&gt;T</td>
<td>18/44(T)</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>c.1084–49G&gt;C</td>
<td>19/44(C)</td>
<td>Y</td>
</tr>
<tr>
<td>12</td>
<td>c.1084–29A&gt;G</td>
<td>12/44(G)</td>
<td>Y</td>
</tr>
<tr>
<td>13</td>
<td>c.1226–20A&gt;G</td>
<td>14/44(G)</td>
<td>Y</td>
</tr>
<tr>
<td>18</td>
<td>c.1715–82T&gt;C</td>
<td>16/44(C)</td>
<td>Y</td>
</tr>
<tr>
<td>19</td>
<td>c.1777–297C&gt;T</td>
<td>7/44(C)</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>c.1826–151T&gt;C</td>
<td>9/44(C)</td>
<td>Y</td>
</tr>
<tr>
<td>20</td>
<td>c.1826–30insG</td>
<td>7/44</td>
<td>Y</td>
</tr>
<tr>
<td>25</td>
<td>c.2014+42G&gt;T</td>
<td>8/44(T)</td>
<td>Y</td>
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<tr>
<td>25</td>
<td>c.2015–71G&gt;A</td>
<td>2/44(A)</td>
<td>N</td>
</tr>
<tr>
<td>25</td>
<td>c.2316+67A&gt;G</td>
<td>2/44(G)</td>
<td>N</td>
</tr>
<tr>
<td>25</td>
<td>c.2316+96G&gt;T</td>
<td>1/44(T)</td>
<td>N</td>
</tr>
<tr>
<td>26</td>
<td>c.2602–36G&gt;T</td>
<td>1/44(T)</td>
<td>Y</td>
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<tr>
<td>26</td>
<td>c.2602–45T&gt;A</td>
<td>1/44(A)</td>
<td>N</td>
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<td>27</td>
<td>c.2778–55G&gt;T</td>
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<tr>
<td>27</td>
<td>c.2779–77C&gt;C</td>
<td>5/44(C)</td>
<td>N</td>
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<tr>
<td>30</td>
<td>c.3067–55A&gt;G</td>
<td>15/44(G)</td>
<td>N</td>
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<td>30</td>
<td>c.3067–42G&gt;A</td>
<td>12/44(A)</td>
<td>Y</td>
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<td>30</td>
<td>c.3067–26G&gt;A</td>
<td>6/44(A)</td>
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<tr>
<td>30</td>
<td>c.3067–42G&gt;A</td>
<td>12/44(A)</td>
<td>Y</td>
</tr>
<tr>
<td>34</td>
<td>c.3084+50G&gt;A</td>
<td>6/44(A)</td>
<td>Y</td>
</tr>
<tr>
<td>35</td>
<td>c.3513+62C&gt;T</td>
<td>8/44(T)</td>
<td>Y</td>
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<tr>
<td>37</td>
<td>c.3765–37A&gt;G</td>
<td>6/44(G)</td>
<td>N</td>
</tr>
<tr>
<td>38</td>
<td>c.3829–82C&gt;G</td>
<td>6/44(G)</td>
<td>N</td>
</tr>
<tr>
<td>38</td>
<td>c.3935–16C&gt;T</td>
<td>6/44(T)</td>
<td>Y</td>
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<tr>
<td>39</td>
<td>c.3935–102C&gt;T</td>
<td>1/44(T)</td>
<td>N</td>
</tr>
<tr>
<td>42</td>
<td>c.4260–29C&gt;T</td>
<td>24/44(C)</td>
<td>Y</td>
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</table>

*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/jumpa.html. DNA numbering is based on the cDNA sequence. The GenBank reference sequence and version number NW_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.

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rate and increased likelihood of cancer development. However, it has been difficult to demonstrate that heterozygote carriers of most FANC gene mutations have an increased risk of developing cancer, 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.

However, the results of this study, our previous study of FANCC and FANC G in familial pancreatic cancer, and the lack of germline mutations in Fanconi genes in familial breast cancers families suggest that any contribution of heterozygote germline FANC gene mutations to cancer predisposition is likely to be a modest one.

Recent work (Kern and coworkers) confirms that pancreatic cancer cells with inactivation of Fanconi genes are hypersensitive to mitomycin C. 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 (FANC D1) as well as to those with FANC G inactivation due to epigenetic silencing by DNA methylation.

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ELECTRONIC-DATABASE INFORMATION


AUTHORS' AFFILIATIONS

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REFERENCES


