

BRCA2 Germline Mutations in Familial Pancreatic Carcinoma

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Background: Although as many as 10% of pancreatic cancer cases may have an inherited component, familial pancreatic cancer has not been linked to defects in any specific gene. Some studies have shown that families with germline mutations in the breast cancer susceptibility gene BRCA2 have an increased risk of breast and ovarian cancers, as well as a modestly increased risk of pancreatic cancer. To study these relationships in more detail, we examined whether BRCA2 germline mutations are associated with familial pancreatic cancer. **Methods:** We identified 26 European families in which at least two first-degree relatives had a histologically confirmed diagnosis of pancreatic ductal adenocarcinoma. We sequenced genomic DNA isolated from peripheral blood lymphocytes obtained from participating family members to identify germline mutations in BRCA2. **Results:** Three (12%, exact 95% confidence interval [CI] = 2% to 30%) families carried germline frameshift mutations in the BRCA2 gene that are predicted to result in a truncated BRCA2 protein. Two additional families harbored mutations previously designated as unclassified variants of BRCA2. Thus, 19% (exact 95% CI = 7% to 39%) of the families in our study had either a frameshift mutation or an unclassified variant of BRCA2. None of the families in our study met the criteria for familial breast or ovarian cancer. **Conclusions:** Our data support an important role for BRCA2 germline mutations in a subpopulation of families with familial pancreatic cancer. BRCA2 mutation analysis should be included in molecular genetic testing and counseling strategies in families with at least two first-degree relatives affected with ductal adenocarcinoma of the pancreas. [J Natl Cancer Inst 2003;95:214-21]

The majority of pancreatic cancer cases are sporadic (i.e., nonhereditary). The existence of familial pancreatic cancer was initially suggested by case reports of familial aggregation of pancreatic ductal adenocarcinoma. The first systematic study of a large cohort of families with pancreatic carcinoma was published in 1989 (1). Since then, it has been estimated that as many

as 10% of pancreatic cancer patients may have an inherited form of the disease, although conclusive epidemiologic data are still lacking (2,3).

The term familial pancreatic cancer is applied to families with at least two first-degree relatives with pancreatic ductal adenocarcinoma but who do not fulfill the criteria for other familial cancer syndromes. In a recent prospective study, the risk for developing pancreatic carcinoma among first-degree relatives of a pancreas cancer patient was estimated to be 18-fold in kindreds with two affected family members, and as high as 57-fold in kindreds with three or more affected family members (2).

Three tumor suppressor genes coding for p53 (TP53), for cyclin-dependent kinase inhibitor 2A (CDKN2A; also called p16^{INK4a}), and for the protein deleted in pancreatic carcinoma, locus 4 (DPC4; also called MADH4), are inactivated in 50%–100% of sporadic pancreatic ductal adenocarcinomas (4). Studies that have tested the hypothesis that the observed familial aggregation of pancreatic carcinomas may be caused by germ-

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See "Notes" following "References."

See "Appendix" for the names of the members of the *German Familial Pancreatic Cancer (FaPaCa) study group of the Deutsche Krebshilfe* and the *EUROPAC study group*.

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line mutations in one of these sporadic pancreatic carcinoma-associated genes have so far not found that families with multiple pancreatic carcinoma cases only have inactivating mutations in these genes (5).

A number of syndromes, including familial atypical multiple mole melanoma (FAMMM) (6,7), Peutz-Jeghers syndrome (PJS) (8–10), hereditary pancreatitis (11), hereditary nonpolyposis colorectal carcinoma (12), familial breast and ovarian cancer (13), and familial adenomatous polyposis (14), are associated with an increased risk for pancreatic carcinoma. The lifetime risks for pancreatic cancer are 40% for those with hereditary pancreatitis (including the form of the disease that is associated with mutations in the cationic trypsinogen gene PRSS1) (11), 36% for those with PJS (10), and 17% for those with FAMMM (6,7). The latter two syndromes are associated with germline mutations in the STK11/LKB1 and CDKN2A genes, respectively, which are also inactivated in sporadic pancreatic carcinoma (15–19). For the remaining syndromes, including breast-ovarian cancer syndromes, the estimated lifetime risk for pancreatic carcinoma is thought to be low (i.e., $\leq 5\%$) with a preference for BRCA2 mutation carriers over BRCA1 carriers (5).

The cloning of the breast cancer susceptibility gene BRCA2 was aided by the identification of a homozygous deletion at 13q12.3 in a pancreatic carcinoma by Schutte et al. (20). Therefore, BRCA2 was considered to be a candidate tumor suppressor gene not only for early-onset breast carcinoma but also for pancreatic carcinoma. Surprisingly, the same group detected no somatic genetic alterations, other than the above-mentioned homozygous deletion, in sporadic pancreatic cancers. However, Goggins et al. (21) found two different BRCA2 germline mutations in two of 30 cases of pancreatic carcinoma, both of which occurred in Ashkenazi Jews. In neither case was there a family history of pancreatic carcinoma, and therefore both cases were considered to be sporadic pancreatic carcinomas (21). Ozelik et al. (22) reported germline BRCA2 mutations in two (4.9%) of 41 unselected patients with pancreatic cancer, including a 6174delT mutation in an Ashkenazi Jewish patient; they subsequently analyzed 39 Ashkenazi Jewish patients with pancreatic cancer and found 6174delT mutations in four (10%) patients. Again, none of these patients had a family history of pancreatic carcinoma. To date, the vast majority of the BRCA2 germline mutations associated with sporadic pancreatic cancers have been found in patients of Ashkenazi Jewish descent; the most common such mutation, 6174delT, occurs in approximately 1% of all Ashkenazi Jews (23).

These studies pointed to the possibility that germline mutations in the BRCA2 gene might play a role in familial pancreatic carcinoma. However, two studies did not identify BRCA2 germline mutations in individuals with familial pancreatic cancer and without a history of breast cancer (2,24). By contrast, one case report describes a BRCA2 germline mutation in a pancreatic cancer family that is not suspected of having familial breast cancer (25). While our manuscript was under review, a study by Murphy and colleagues (26) was published that reports on BRCA2 germline mutations in five (17%) of 29 families with three or more relatives with pancreatic cancer. Of note, three of the five families were of Ashkenazi Jewish descent and harbored the common 6174delT frameshift mutation previously found only in sporadic cases of pancreatic carcinoma (21,22).

To gain more insight into the role of BRCA2 mutations in familial pancreatic cancer, we analyzed the frequency of

BRCA2 germline mutations in 26 European families in which at least two first-degree relatives were diagnosed with histologically confirmed pancreatic ductal adenocarcinoma. All of the families in our study identified themselves as white, and none was of Ashkenazi Jewish descent. In addition, none of the families fulfilled the clinical criteria for other known hereditary tumor syndromes.

SUBJECTS AND METHODS

Selection of Families

We used two sources to identify families with familial pancreatic cancer: the German National Case Collection for Familial Pancreatic Carcinoma of the German Cancer Foundation (FaPaCa) (27,28) and the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) (29). Since July 1999, families with at least two first-degree relatives with histologically confirmed pancreatic ductal adenocarcinoma were collected in FaPaCa. A genetic counselor from the FaPaCa group verified the clinical diagnoses reported by patients and family members by consulting the original medical and tumor histology records provided by the clinics and personal physicians that initially diagnosed the patients. Since 1997, EUROPAC has included families that have at least two first-degree relatives with pancreatic ductal adenocarcinoma, at least three relatives of any degree with pancreatic ductal adenocarcinoma, or any two relatives with pancreatic ductal adenocarcinoma if the sum of their ages at diagnosis was less than 110 years. A genetic counselor from EUROPAC confirmed the diagnosis of pancreatic ductal adenocarcinoma whenever possible by reviewing one or more of the following: tumor histology, medical records, cancer registry data, or family history. All families included in FaPaCa and EUROPAC were genetically counseled by a genetic counselor from the respective study group. We obtained written informed consent from each family member who was interviewed or who provided blood and/or tissue. On the basis of personal interviews with family members, we prepared a complete three-generation pedigree for each family that included data about its medical history, all tumors, and the age of each patient at the onset of cancer. The study protocols were approved by the Ethics Committee of the Philipps-University of Marburg in Marburg, Germany, and the Liverpool Research Ethics Committee in Liverpool, U.K.

When we initiated our analysis of BRCA2 mutations, the FaPaCa registry had collected 19 familial pancreatic cancer families and the EUROPAC registry had collected 39 such families. For the present study, we applied the following family selection criteria: 1) at least two first-degree relatives had to be affected with histologically confirmed pancreatic ductal adenocarcinoma and 2) DNA sufficient for germline mutation analysis had to be available from at least one affected family member with pancreatic cancer. Twenty-seven families from the EUROPAC registry were excluded because histologic confirmation of the pancreatic cancer in at least two patients from each family was not available. Five families from the FaPaCa registry were excluded because no DNA was available from any family member affected with pancreatic cancer. Thus, 26 families, 12 from the EUROPAC registry and 14 from the FaPaCa registry, fulfilled the selection criteria for our study. None of these 26 families were of Ashkenazi Jewish descent. On the basis of the medical history obtained from each personally interviewed family mem-

ber, none of these pancreatic cancer families fulfilled the published criteria for other hereditary cancer syndromes.

BRCA2 Mutation Analysis

Two separate blood samples for DNA analysis were collected from participating family members by the genetic counselors after the counseling procedure. We isolated genomic DNA from peripheral blood lymphocytes in those samples with the use of the QIAamp DNA kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Isolated DNA was stored at -20°C . We used that DNA as a template to sequence the entire BRCA2 gene, including all exon-intron borders, from at least one member of each family, as follows. First, we used polymerase chain reaction (PCR) to amplify each of the 26 coding exons of the BRCA2 gene. Exons 10 and 11 were amplified as overlapping fragments, resulting in a PCR product with a maximal fragment size of 650 base pairs (bp). PCR was performed in a final reaction volume of 15 μL that contained 10–100 ng of genomic DNA in 67 mM Tris-HCl (pH 8.8), 4 mM MgCl_2 , 16 mM $(\text{NH}_4)_2\text{SO}_4$, 10 mM 2-mercaptoethanol, 100 g/mL bovine serum albumin, dATP, dCTP, dGTP, and dTTP at 200 μM , each primer at 1 μM , and 2 U of *Taq* polymerase (Invitrogen, Karlsruhe, Germany). The polymerase was added after the other reaction components were preheated for 2 minutes at 94°C . PCR then consisted of five cycles at 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 1 minute, followed by 35 cycles at 94°C for 30 seconds and 70°C for 1 minute. Each PCR product (1.5 μL) was directly sequenced without prior purification using the DYEnamic cycle sequencing system (Amersham Pharmacia, Freiburg, Germany), according to the manufacturer's protocol. Sequencing reactions were analyzed with a LICOR 4200 LR sequencer (MWG Biotech, Ebersberg, Germany). The sequences of the primers used for PCR and for sequencing are available online at the Journal's Web site available at <http://jncicancerspectrum.oupjournals.org/jnci/content/vol95/issue3/index.shtml>. Each BRCA2 mutation was confirmed by bidirectional sequencing of an independent PCR product derived from a separately drawn blood sample.

Statistical Analysis

The 95% confidence intervals (CIs) for the fraction of families with frameshift mutations and the fraction of families with frameshift mutations or unclassified variants were calculated with the use of StatXact 5 for Windows, version 2001 (CYTEL Software Corp., Cambridge, MA).

RESULTS

Families

There were 64 patients (37 men and 27 women) with pancreatic cancer among the 26 families in our study. Four families had four affected members with pancreatic cancer, four families had three affected members, and 18 families had two affected members. Three families had three affected generations, 16 families had two affected generations, and seven families had only one affected generation. The median age at diagnosis was 60 years (range = 33–81 years); 14 (22%) patients were younger than 50 years at diagnosis. Five families had at least one member diagnosed with breast cancer; three families had at least one member diagnosed with colon cancer; two families had at least one member diagnosed with prostate, gastric, or lung can-

cer; and four families had one member diagnosed with either head and neck, esophageal, or ovarian cancer or osteosarcoma.

BRCA2 Mutation Analysis

The results of the mutation analysis are summarized in Table 1. Three of the 26 families (i.e., families N1, 2-8-27, and 25-7-1) carried a frameshift mutation in the BRCA2 gene. Family 2-8-27 carried mutation 6672insT, an insertion of T at position 6672; family 25-7-1 carried mutation 6819delTG, a deletion of T and G at position 6819; and family N1 carried mutation 4075delGT, a deletion of G and T at position 4075 (Fig. 1). Each of these mutations is predicted to produce a truncated, nonfunctional Brca2 protein. Thus, 12% (exact 95% CI = 2% to 30%) of the families in our study had frameshift mutations.

The pedigrees of these three families are depicted in Fig. 2. Family N1 had three first-degree relatives with pancreatic ductal adenocarcinoma and family 2-8-27 had two first-degree relatives with pancreatic ductal adenocarcinoma. Family 2-8-27 had no other known tumor types. In family N1, a suspected ovarian carcinoma occurring in a woman at the age of 26 years (Patient 06), a suspected colon cancer, one gastric cancer, and two cancers of unclear origin were reported. Sequence analysis revealed that Patient 06 in family N1 did not carry the germline BRCA2 mutation that was carried by the pancreatic cancer patient (Patient 02) in this family. Family 25-7-1 had four first-degree relatives with pancreatic cancer. DNA from two of those affected members (Patient 2 and Patient 206) was available for mutation analysis; both individuals had the 6819delTG mutation. Patient 204 from this family developed ductal adenocarcinoma of the breast at the age of 47 years; 6 years later she developed pancreatic ductal adenocarcinoma. Additional tumor types reported in this family were one prostate carcinoma, two carcinomas of unclear origin, one osteosarcoma, and two colorectal carcinomas. We did not observe a pattern consistent with hereditary breast or ovarian cancer in any of these three families.

We identified sequence variants of BRCA2 in two of the 26 families in our study. Family N2 carried the R2034C substitution in which the arginine at amino acid 2034 of Brca2 was replaced with a cysteine due to a C→T transition at position 6328. In this family, two first-degree relatives had pancreatic ductal adenocarcinoma, but no other tumor types were reported among first-degree relatives. DNA for sequence analysis was available from the index patient only. Family 9-3-26 carried two sequence variants, G3076E and 10323delCins11, presumably on the same allele of BRCA2 (Fig. 1). In the G3076E variant, the glycine at amino acid 3076 of Brca2 was replaced with a glutamic acid due to a G→A transition at position 9455, and in the 10323delCins11 variant, a C was deleted and 11 bases were inserted at position

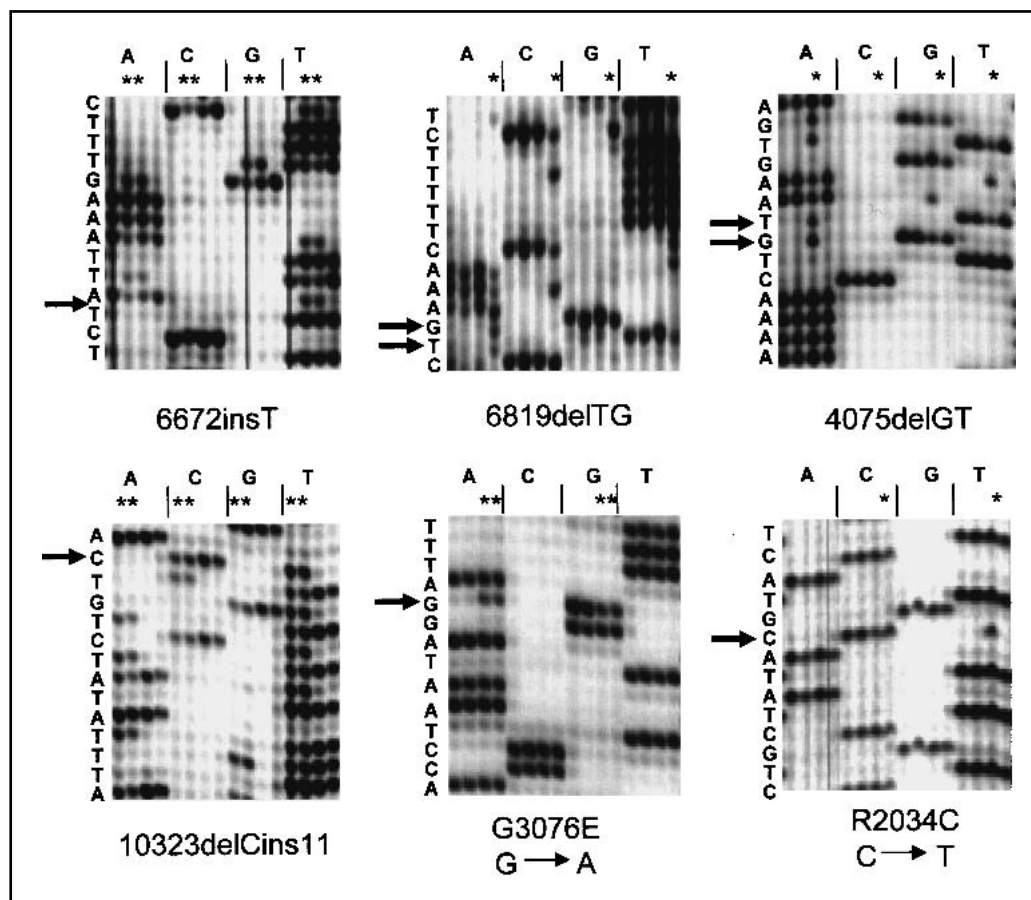
Table 1. Mutations and unclassified variants in the BRCA2 gene*

Family	Nucleotide change	Exon	Mutation type	In BIC database
N1	4075delGT	11	FS	Yes
2-8-27	6672insT	11	FS	Yes
25-7-1	6819delTG	11	FS	Yes
N2	R2034C	11	MS/UV	Yes
9-3-26†	G3076E	24	MS/UV	Yes
	10323delCins11	27	FS/UV	Yes

*BIC = Breast Cancer Information Core; FS = frameshift; MS = missense; UV = unclassified variant.

†Family with two unclassified variants identified on one allele.

Fig. 1. BRCA2 sequencing results. For BRCA2 mutations 6672insT, 10323delCins11, and G3076E, sections of representative sequencing gels are shown where samples from different family members that did and did not carry the mutations were loaded. For the remaining mutations, sequencing gels are shown where samples from patients with a mutation were loaded along with samples from patients from different families which were not carrying the mutation. Four sequencing reactions were grouped by termination dideoxynucleotide to aid pattern recognition. **Arrow** indicates location of the indicated sequence alterations in the BRCA2 gene; **star** indicates lanes that contain DNA samples from individuals with BRCA2 mutations.



10323. To date, three first-degree relatives in family 9-3-26 (Patient 2, Patient 202, and Patient 209) have developed pancreatic ductal adenocarcinoma (Fig. 2). In addition to pancreatic ductal adenocarcinoma, Patient 2 and Patient 202 had ductal adenocarcinomas of the breast. Patient 204 had a breast carcinoma only. When this family was identified in 1999, Patient 2 and her brother (Patient 209) had developed pancreatic cancer at the ages of 46 and 50 years, respectively, and their mother (Patient 202) had developed a breast carcinoma at the age of 74 years. During the next 30 months of follow-up, Patients 2 and 204 developed breast cancer at the ages of 51 and 52 years, respectively, and Patient 202 developed pancreatic cancer at the age of 75 years. Additional tumors reported in this family were one lung carcinoma, one gastric carcinoma, and one cancer of unknown origin. The two sequence variants of BRCA2 were identified in Patients 2, 202, and 204 (no DNA for sequence analysis was available from Patient 209). Thus, 19% (exact 95% CI = 7% to 39%) of the families in our study had either a frameshift mutation or an unclassified variant of BRCA2.

Smoking is thought to have an influence on the phenotypic expression of familial pancreatic carcinoma. We therefore examined the data on smoking history that was available for affected patients in the families that carried sequence changes in BRCA2. Both patients with pancreatic cancer in family 2-8-27 and three of the pancreatic cancer patients in family 25-7-1 (Patients 2, 204, and 206) were nonsmokers. Patient 02 in family N1 smoked four cigars per day for approximately 10 years; the smoking habits of the two other pancreatic cancer patients in this family are unknown. In family 9-3-26, Patient 202, who developed pancreatic cancer at the age of 75 years, was a nonsmoker,

whereas Patient 2, who developed pancreatic cancer at the age of 46 years, smoked 5–10 cigarettes per day from the age of 18 years. Patient 204 in family 9-3-26, who developed breast cancer at the age of 52 years, smoked 10 cigarettes per day between the ages of 30 and 40 years. The smoking habits of the two pancreatic cancer patients in family N2 are unknown.

DISCUSSION

An inherited predisposition to pancreatic cancer is currently believed to occur in three distinct clinical settings. The first setting is familial cancer syndromes, including PJS, FAMMM, familial breast–ovarian cancer, hereditary nonpolyposis colorectal carcinoma, and familial adenomatous polyposis, that are associated with an increased risk for pancreatic cancer (5). The second setting is hereditary pancreatitis, although in this instance, germline mutations of the cationic trypsinogen (PRSS1) gene are linked only indirectly to pancreatic cancer through the early onset of chronic pancreatitis (11). The third setting is in familial pancreatic cancer, in which two or more first-degree relatives have pancreatic cancer without fulfilling the criteria for one of the aforementioned cancer syndromes.

We report the BRCA2 germline mutation status of 26 families with familial pancreatic cancer in which the diagnosis of pancreatic carcinoma was histologically confirmed in at least two affected family members. The median age at diagnosis of family members with pancreatic cancer was 60 years, which is about 8–10 years younger than the median age at diagnosis of sporadic pancreatic cancer for Europeans (30). Twenty-two percent of the affected patients were younger than 50 years (the youngest was 33 years old) at the time of diagnosis.

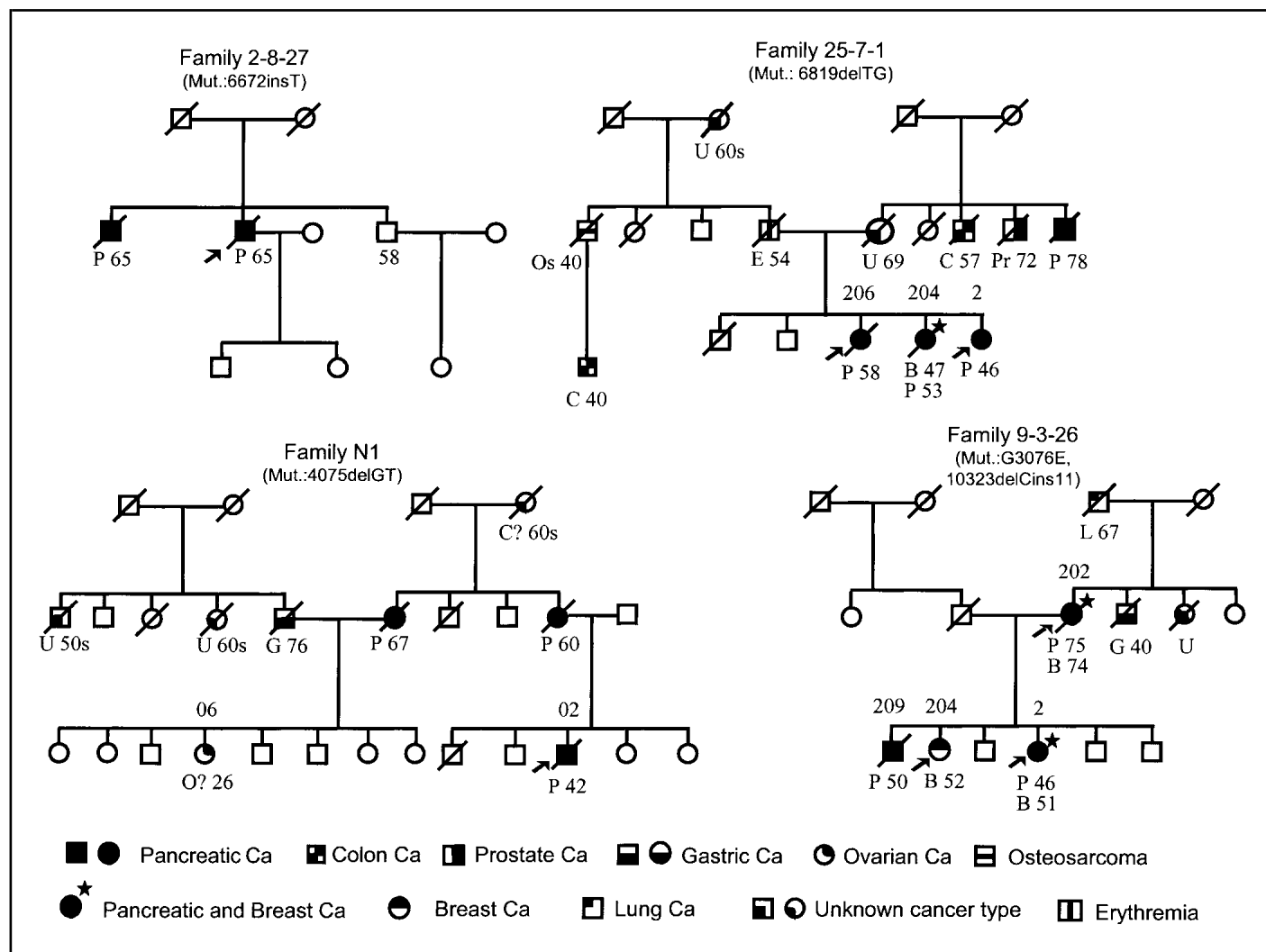


Fig. 2. Pedigrees of the families with familial pancreatic cancer that carried BRCA2 germline mutations. The specific mutation(s) carried by each family is listed under the family name. The number under the symbol indicates the age at diagnosis of the disease. The number above the symbol represents a patient identifier referred to in the text. **Arrow** indicates patients with BRCA2 mutations

demonstrated by DNA sequence analysis. P, pancreatic carcinoma; C, colon carcinoma; Pr, prostate carcinoma; G, gastric carcinoma; O, ovarian carcinoma; B, breast carcinoma; L, lung carcinoma; Os, osteosarcoma; E, erythremia; U, unknown tumor type; Ca, carcinoma.

We included families from Germany and the United Kingdom in our study. Therefore, one might ask whether mixing these two population pools in one study could create a potential selection bias. The results of recent empirical work suggest that Europe can be considered to comprise a single genetic pool (31), so that our combination of case patients collected from two different European countries is not expected to cause such a bias.

Using sequencing analysis, we found BRCA2 germline frameshift mutations (4075delGT, 6672insT, and 6819delTG) in three families; all three mutations were located within exon 11. These mutations are considered to be pathogenic, because each creates a premature stop codon that is predicted to cause a truncation of the Brca2 protein. The predilection for such frameshift mutations in exon 11 in these patients is similar to the type and location of BRCA2 mutations that are deposited in the breast cancer information core (BIC) database, as well as to that among a large series of German families with familial breast and ovarian cancer (32).

We also identified three sequence variants of BRCA2 among the families in this study. All three variants were deposited in the BIC database as unclassified variants. One of those variants,

10323delCins11, was previously found in a cohort of 777 familial breast-ovarian carcinoma patients who were analyzed for mutations in BRCA2 through the German Breast Cancer Consortium (32). The pathogenic roles of these variants cannot be predicted in the absence of functional tests for BRCA2 or models of protein folding that are linked to function. Two of the three unclassified sequence variants (10323delCins11 and G3076E) were identified in family 9-3-26, in which there were three pancreatic and three breast cancers, with two patients having both cancer types. All three mutations with breast and/or pancreatic cancer in this family harbored both BRCA2 sequence variants. The high incidence of both pancreatic and breast carcinoma and the relative young age at onset of pancreatic carcinoma in this family suggest that either one of the BRCA2 sequence variants alone or both of the variants together are equally likely to predispose to both pancreatic and breast cancer. In addition, both sequence variants found in this family likely resided on the same allele of BRCA2, because we found that they co-segregated over two generations. Although we were not able to formally exclude the possibility that the husband of Patient 202 also carried one of those two variants, the lack of detection of one of these variants

alone and the very low frequency of detection of the other variant reported from 777 German familial breast-ovarian carcinoma patients makes this possibility highly unlikely (32).

An important finding of our study was that, in general, the pancreatic carcinoma families in our study did not fulfill the criteria for having hereditary breast and ovarian cancer or any other known tumor syndrome. The one possible exception might be family 9-3-26, which had three first-degree relatives with breast carcinomas. However, all of the three breast carcinomas reported were diagnosed in individuals who were older than 50 years. Currently it is unclear whether families with two or more members diagnosed with breast carcinomas after the age of 50 years should be classified as families with familial breast cancer. In a series of 45 families with these criteria (i.e., fewer than two breast cancers diagnosed in members older than 50 years of age), only three families with truncating BRCA2 mutations and four with unclassified BRCA2 variants were reported, suggesting that only a minor fraction of such cases are familial (32).

Previously published data suggest that BRCA2 germline mutation carriers may exhibit at least two different cancer phenotypes (21,33). The first cancer phenotype, represented by families with BRCA2 mutations that have a preponderance of breast and ovarian carcinomas, can be further classified according to the occurrence of pancreatic cancer. The second cancer phenotype is represented by the minority of patients with pancreatic cancer that have germline BRCA2 mutations but no history of familial pancreatic or breast cancer (these cases are therefore called "sporadic"). Our results and those of Murphy et al. (26) suggest that a third cancer phenotype exists, namely that a proportion of familial pancreatic cancers is caused by BRCA2 germline mutations. Of note, these pancreatic cancer families generally do not show an increased incidence of breast and ovarian cancer.

We currently do not understand what causes these phenotypic variations observed in BRCA2 germline mutation carriers. One explanation might be that one or several modifier genes suppress or induce the pancreatic cancer or breast cancer phenotype to a varying degree in BRCA2 mutation carriers. Interestingly, inactivation of the second BRCA2 allele appears to occur relatively late in the molecular evolution of the sporadic pancreatic cancers in individuals who carry a BRCA2 germline mutation (34). This finding may explain why BRCA2 germline mutations in these patients have a very low penetrance and why some BRCA2 mutation carriers have a late onset of pancreatic cancer. In light of this observation, it would be important to analyze cancers of family members diagnosed with familial pancreatic cancer and known BRCA2 germline mutations for differences in the timing of biallelic inactivation of the BRCA2 gene. We speculate that one explanation for the apparently higher prevalence of pancreatic carcinoma in families with two or more affected first-degree BRCA2 germline mutation carriers (families fulfilling the current clinical criteria for familial pancreatic carcinoma), could be due to the early biallelic inactivation of the BRCA2 gene, which would fit into the "caretaker" model for tumor suppressor genes that has been suggested by others (35,36). If our speculation is correct, the phenotypic prevalence of pancreatic carcinoma among some BRCA2 mutation carriers could be explained by the early inactivation of BRCA2 combined with the expression of modifying genes that partly or completely suppress the breast-ovarian cancer phenotype.

The principal environmental risk factor for pancreatic cancer

is tobacco smoking. Several studies (2,3,37) have so far failed to find a statistically significant link between smoking and a family history of pancreatic cancer. However, one study (38) found that the average age at which smokers developed pancreatic cancer was 10 years younger than the average age at which nonsmokers developed the disease, which suggests that smoking can increase the risk of pancreatic cancer in BRCA2 mutation carriers who have a known genetic predisposition for this disease. This hypothesis can now be tested during follow-up of pancreatic cancer families with associated BRCA2 mutations. Further studies are in progress to identify other genes that may be linked to familial pancreatic cancer (39).

Our study is the first to analyze a relatively large group of European families with familial pancreatic cancer for BRCA2 mutations. Two previously published studies from the United States (2,24) did not detect BRCA2 germline mutations in families with two or more affected first-degree relatives. Both studies used less stringent criteria than our study to define familial pancreatic cancer. One study (24) included families with pancreatic cancer in second- and third-degree relatives. The other study (2) did not consistently verify the diagnosis of pancreatic ductal adenocarcinoma by histology. We have found that a positive family history for pancreatic cancer reported by index patients may be incorrect in up to 30% of cases (27). Thus, a stringent method, i.e., histologic confirmation, for confirming the diagnosis of pancreatic carcinoma appears to be important for identifying true cases of familial pancreatic carcinoma. However, one drawback of using such a stringent selection criterion is that many families with at least two first-degree relatives with pancreatic cancer were excluded from our study because they lacked histologic confirmation. Therefore, it remains unclear whether the frequency of germline BRCA2 mutations that we have reported is representative for all families with familial pancreatic cancer. Nevertheless, our use of stringent inclusion criteria is important to avoid the bias caused by incorrectly including families with pancreatic cancers other than pancreatic ductal adenocarcinomas, i.e. peri-pancreatic tumors.

While this manuscript was under review, Murphy et al. (26) reported on BRCA2 germline mutations in five (17%) of 29 families with suspected familial pancreatic carcinoma. Of those 29 families, six were of Ashkenazi Jewish descent, and three of those six families carried the common 6174delT mutation (22,23). By contrast, our study included only European families of non-Jewish descent and, therefore, the reported frequency of BRCA2 germline mutation in our study is likely to reflect the frequency for white non-Jewish Europeans and may also apply to the non-Jewish white population of European descent in the United States. The study by Murphy et al. (26) included only families with three or more cases of pancreatic carcinoma where at least two of the affected persons were first-degree relatives. By contrast, our study included families with two or more first-degree relatives with pancreatic cancer. This strategy led to the discovery of a BRCA2 germline mutation in one family (2-8-27) with only two first-degree relatives with pancreatic cancer. Clearly, family size strongly influences the ability to identify more than two pancreatic carcinoma cases in families with familial pancreatic carcinoma, as was required in the study of Murphy et al. (26). Unfortunately, in many cases, reliable information on family members over several generations is not available. In light of this potential limitation, our finding of a BRCA2 germline mutation in a family with only two first-degree rela-

tives with pancreatic cancer suggests that families with as few as two first-degree relatives with pancreatic carcinoma should be included in a BRCA2 mutation screening.

Our study shows that germline BRCA2 mutations are associated with the predisposition for development of pancreatic cancer in 12%–19% of European families with familial pancreatic cancer. This finding has immediate implications for the genetic counseling and clinical screening of individuals from kindreds with at least two first-degree relatives affected with histologically confirmed ductal adenocarcinoma of the pancreas.

APPENDIX

German Familial Pancreatic Cancer (FaPaCa) study group of the Deutsche Krebshilfe: S. A. Hahn, M. Sina-Frey, H. Rieder, B. Korte, B. Gerdes, R. Kress, A. Ziegler, R. Grützmann, H. Rehder, M. Rothmund, W. Schmiegell, D. K. Bartsch.

EUROPAC study group: B. Greenhalf, I. Ellis, J. A. Raeburn, D. Campra, J. P. Neoptolemos.

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NOTES

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