Evaluation of Fanconi Anemia Genes in Familial Breast Cancer Predisposition

Sheila Seal,1 Rita Barfoot,1 Hiran Jayatilake,1 Paula Smith,2 Anthony Renwick,1 Linda Bascombe,1 Lesley McGuffog,2 D. Gareth Evans,3 Diana Eccles,4 The Breast Cancer Susceptibility Collaboration (UK), Douglas F. Easton,2 Michael R. Stratton,1,2 and Nazneen Rahman1

1Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey; 2Cancer Research UK Genetic Epidemiology Unit, Strangeways Research Laboratories, University of Cambridge, Cambridge; 3Department of Medical Genetics, St Mary's Hospital, Manchester; 4Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton; and 5Cancer Genome Project, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambs, United Kingdom

Abstract

Fanconi Anemia (FA) is an autosomal recessive syndrome characterized by congenital abnormalities, progressive bone marrow failure, and susceptibility to cancer. FA has eight known complementation groups and is caused by mutations in at least seven genes. Biallelic BRCA2 mutations were shown recently to cause FA-D1. Monoallelic (heterozygous) BRCA2 mutations confer a high risk of breast cancer and are a major cause of familial breast cancer. To investigate whether heterozygous variants in other FA genes are high penetrance breast cancer susceptibility alleles, we screened germ-line DNA from 88 BRCA1/2-negative families, each with at least three cases of breast cancer, for mutations in FAANCA, FANCC, FANCD2, FANCE, FANCF, and FANCJ. Sixty-nine sequence variants were identified of which 25 were exonic. None of the exonic variants resulted in translational frame shifts or nonsense codons and 14 were polymorphisms documented previously. Of the remaining 11 exonic variants, 2 resulted in synonymous changes, and 7 were present in controls. Only 2 conservative missense variants, 1 in FAANCA and 1 in FANCE, were each found in a single family and were not present in 300 controls. The results indicate that FA gene mutations, other than in BRCA2, are unlikely to be a frequent cause of highly penetrant breast cancer predisposition.

Introduction

Fanconi Anemia (FA) is a rare autosomal recessive syndrome with a prevalence of about 1–5 per million and a heterozygote frequency estimated at 1 in 300 (for all FA disease alleles together) in Europe and the United States. FA is characterized clinically by skeletal abnormalities, skin pigmentary defects, and short stature together with progressive bone marrow failure, cancer susceptibility, and cellular hypersensitivity to DNA cross-linking agents, such as mitomycin C and cisplatin. Somatic cell fusion analyses led to FA cases being assigned into eight distinct complementation groups, FA-A, FA-B, FA-C, FA-D1, FA-D2, FA-E, FA-F, and FA-G. Sixty-nine sequence variants were identified of which 25 were exonic. None of the exonic variants resulted in translational frame shifts or nonsense codons and 14 were polymorphisms documented previously. Of the remaining 11 exonic variants, 2 resulted in synonymous changes, and 7 were present in controls. Only 2 conservative missense variants, 1 in FAANCA and 1 in FANCE, were each found in a single family and were not present in 300 controls. The results indicate that FA gene mutations, other than in BRCA2, are unlikely to be a frequent cause of highly penetrant breast cancer predisposition.

Received 7/4/03; revised 9/19/03; accepted 10/24/03.

Grant support: Cancer Research UK.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Notes: The Breast Cancer Susceptibility Collaboration (UK) members that supplied families: Audrey Ardern-Jones, Rachel Belk, Nicola Bradshaw, Angela Brady, Barbara Bullman, Roseanne Cetnarowy, Cyril Chapman, Trevor Cole, Gillian Crawford, Carol Cummings, Rosemarie Davidson, Alan Donaldson, Diana Eccles, Rosalind Eeles, Graham Evans, Sheila Goff, Jonathon Gray, Helen Gregory, Neva Haite, Shirley Hodgson, Tessa Homfray, Richard Houlston, Louise Ilett, Liane Jackson, Lisa Jeffers, Fiona Laloo, Mark Longmair, Donna McIntyre, James Mackay, Alex Magee, Sahar Mansour, Patrick Morrison, Vicky Murtlay, Joan Paterson, Mary Porteous, Nazneen Rahman, Mark Rogers, Andy Schofield, Sue Shanley, Janet Shea-Simmonds, and Lesley Snaddon; Supplementary data for this article are available at Cancer Research Online (http://cancercres.aacrjournals.org).

Requests for reprints: Michael R. Stratton, The Cancer Genome Project, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom. Phone: 44-1223-494-951; Fax: 44-1223-494-969; E-mail: mrs@sanger.ac.uk.

The proteins encoded by FA genes are intimately related to each other in molecular pathways involved in DNA repair, and FANCA, FANCC, FANCE, FANCJ, and FANCG interact directly to form a multisubunit nuclear complex (3). In response to DNA damage this complex is translocated to DNA repair foci containing BRCA1 and BRCA2 (4). The convergence of FA gene and BRCA1/BRCA2 biological pathways (5), the fact that cell lines homozygous for BRCA1 or BRCA2 mutations are hypersensitive to mitomycin-C (6, 7), and the observation that homozygous BRCA2 mutant mice have phenotypic features similar to FA (8), led Howlett et al. (9) to screen FA cases without mutations in known FA genes for mutations in BRCA1 and BRCA2. One FA-B and two unassigned FA cases were each heterozygous for truncating BRCA2 mutations. A second BRCA2 sequence variant of unknown significance was identified in each case (9). The reference FA-D1 cell line was homozygous for a BRCA2 splicing mutation that results in an in-frame deletion of four amino acids, and an additional FA-D1 case carried two truncating BRCA2 mutations (9). Overall, these data suggest strongly that the gene causing FA-D1 is BRCA2. This is additionally supported by the observation of partial rescue of sensitivity to mitomycin C of a FA-D1 cell line by introduction of wild-type BRCA2 (9).

The majority of site-specific breast cancer families with three, four, or five cases diagnosed earlier than age 60 are not due to BRCA1 or BRCA2 (10). Moreover, no more than 20% of the familial risk of breast cancer is accounted for by currently recognized breast cancer susceptibility genes, BRCA1, BRCA2, TP53, PTEN, ATM, and CHEK2 (11). Therefore, the identification of BRCA2 mutations in FA-D1 patients has fostered speculation that heterozygotes for mutations in other FA genes may have a high risk of breast cancer, similar to that seen in BRCA2 heterozygotes. Homozygotes for FA gene mutations are clearly at increased risk of cancer, because FA patients often develop acute myeloid leukemia in childhood, and those that survive to adulthood are at increased risk of developing solid tumors, especially hepatic adenomas and squamous cell carcinomas of the esophagus, oropharynx, and vulva (12). Epidemiological studies have not detected increased cancer risks in FA heterozygotes (13, 14). However, these studies have been limited by their small sample sizes and their inability to study individual complementation groups. Hence, anecdotal reports of clustering of cancer cases in FA families and reported nonsignificant increases in bladder, breast cancer, and gastric cancer remain interesting and require additional consideration (13, 15).

To evaluate directly whether sequence variants of FA genes other than FANCD1/BRCA2 might be rare, high-risk breast cancer susceptibility alleles, we have screened the coding sequence and intron-exon
boundaries of all of the FA genes in BRCA1/2-negative breast cancer families from the United Kingdom.

Materials and Methods

Ascertainment of Cases and Controls. Breast cancer families with at least three cases of breast cancer were ascertained as part of the Familial Breast Cancer Study from Clinical Genetics centers in the United Kingdom, with the approval of the London Multi-Research Ethics Committee. Healthy controls were obtained from Human Random Control DNA panels from the European Collection of Cell Cultures (Salisbury, United Kingdom). Controls were Caucasians from the United Kingdom.

Analyses of BRCA1 and BRCA2. DNA was extracted using standard methods. At least one case from each family was screened through the complete coding sequence of BRCA1 and BRCA2 by conformation sensitive gel electrophoresis (16) and was negative. The residual probabilities of carrying a BRCA1 or BRCA2 mutation were evaluated using the model of Antoniou et al. (17). This algorithm models the familial aggregation of breast cancer in terms of mutations in BRCA1 and BRCA2 and an additional “polygenic” component representing the effects of a large number of genes of small effect. The algorithm was implemented in the program Mendel, and the residual probability that the index case in each family carried a mutation was computed conditional on their family history and on their being tested for mutations in BRCA1 and BRCA2. Using this algorithm the expected number of mutation carriers remaining in the set was 4.4 for BRCA1 and 5.9 for BRCA2.

Mutation Analysis of FA Genes. FANCA, FANCC, FANCD2, FANCE, FANCF, and FANCG were screened for small intragenic mutations by conformation sensitive gel electrophoresis. Amplifying primers flanking exons and intron-exon boundaries were designed using the genomic sequence for each gene, and Primer3 software (primers and conditions available on request). The number of exons and fragments screened for each gene is shown in Table 1. Three fragments (FANCD2 exons 14, 20, and 24) produced data that was difficult to interpret even after redesign of the primers, and we consider these exons to be unscreened. The poor quality of these fragments may be due to partial genomic copies of this gene. This problem might conceivably compromise screening other parts of FANCD2, although we were able to detect polymorphisms reported previously and novel variants without apparent difficulty. Complete data from the remaining 125 fragments was obtained from >90% of samples. Genomic DNA from cases showing mobility shifts was sequenced bidirectionally using the BigDye Terminator Cycle Sequencing kit and a 3100 automated sequencer (ABI Perkin-Elmer).

Approximately 40% of FANCA mutations are heterozygous large intragenic deletions. We used a quantitative fluorescent multiplex PCR assay published previously to screen for ~75% of known FANCA deletions (18). In the multiplex, 6-fam-labeled primers for FANCA exons 5, 17, 35, and 43, and FANCC exons 5 and 6 (which were used as controls) were amplified simultaneously by PCR. The resulting products were electrophoresed on an ABI 3100 sequencer (ABI Perkin-Elmer) and analyzed with GENOTYPER software. All of the experiments were repeated six times, and positive controls for FANCA exon 5 and exon 17 deletions were included in all of the experiments. A consistent, reproducible reduction by half in peak height of a FANCA exon compared with the FANCC exons was taken as evidence of a deletion. The quantitative PCR assays, particularly for exon 5, were variably successful, but evaluable data from 79 samples were obtained.

Results and Discussion

DNAs from 88 breast cancer families without BRCA1 or BRCA2 mutations were screened for sequence variants in FANCA, FANCC, FANCD2, FANCE, FANCF, and FANCG. Each family was characterized by at least three cases of breast cancer diagnosed under age 60. At least two (and usually three) of these early onset cases were first- or second-degree relatives. The distribution of breast cancer cases per family was as follows: 8 cases, 1; 7 cases, 3; 6 cases, 5; 5 cases, 8; 5 cases, 18; 4 cases, 28; and 3 cases, 30. The number of breast cancer cases diagnosed under age 60 per family was: 7 cases, 1; 6 cases, 2; 5 cases, 7; 4 cases, 22; and 3 cases, 56.

In total we identified 69 sequence variants in the 88 samples from breast cancer families. Forty-four of the variants were intronic (not involving consensus splice sites) and 7 (all in FANCA) have been reported as polymorphisms, i.e., sequence variants that are not associated with FA (see Supplementary Data).6 On the basis of the likely absence of effect on protein function and the fact that most were seen in multiple individuals, we consider it unlikely that these intronic variants are high-risk breast cancer susceptibility alleles. Therefore, these were not additionally investigated. No large deletions of FANCA were detected in the quantitative multiplex PCR.

Twenty-six exonic variants were identified, but none caused translational frameshift or nonsense codons, and 8 were silent variants. Eighteen exonic variants encoded missense changes. Nine of these were known polymorphisms, 2 have been reported as mutations, and 7 were novel (Table 2). To investigate the 9 missense alterations that were either reported as FA-causing mutations (FANCA S1088F and FANCA H1147D) or were not reported previously (FANCA L1143V; FANCC V60I, FANCC E417L; FANCD2 T896M; FANCE R365K, FANCE A502T; and FANCF P320L) we screened the relevant exons in 300 United Kingdom controls and detected 7 of the variants (Table 2).

Only 2 conservative missense variants, FANCA L1143V and FANCE R365K, were not detected in controls and have not been reported previously as polymorphisms. To additionally assess these 2 variants we examined their segregation with breast cancer in the families in which they were detected. FANCA L1143V was identified in family B637, which consists of twin sisters and two additional sisters all affected with breast cancer before age 55 years. The twins (who are thought to be monozygotic) and one sister carried FANCA L1143V as did an unaffected brother. The status of the fourth sister is unknown. FANCE R365K was identified in family B529, which consists of a mother and two daughters all affected with breast cancer before 60 years of age, all of whom carried the variant.

Our analyses are the first to report mutation screens of FANCD2, FANCE, and FANCF since the original discovery of these genes. We have detected a number of novel sequence variants and have also demonstrated that two variants reported previously as causing FA are unlikely to be classical FA disease-causing mutations. FANCA S1088F was reported as the causative mutation in a consanguineous Middle Eastern FA-A family (19). However, our data show it is a relatively common variant found in healthy individuals and, therefore, perhaps more likely to be a polymorphism. FANCA H11714D has also been reported as a mutation in a Middle Eastern consanguineous

---

6 The Fanconi Anemia Mutation Database, internet address: http://www.rockerfeller.edu/fanconi/mutate.
family (20). However, we identified this variant in two cases and one control, again suggesting that it is a polymorphism. These results emphasize the need for caution in interpretation of missense variants, particularly in consanguineous cases where all of the sequence alterations in and around the relevant gene are likely to be homozygous in affected individuals.

We have not identified any clearly pathogenic FA gene mutations in 88 breast cancer pedigrees. Only 2 missense coding variants, FANCA L1143V and FANCE R365K, were seen in familial breast cancer cases and were absent in controls. Although these variants segregated with breast cancer in the families, the pedigrees are small, and segregation could have occurred by chance. Moreover, both amino acid changes are conservative and, hence, overall are unlikely to be high-penetration breast cancer susceptibility alleles.

In this study, we have investigated whether individual variants of individual FA genes may be high-risk breast cancer susceptibility alleles. Overall, our data suggest that heterozygous FA gene mutations, except for those in BRCA2, do not confer a high risk of breast cancer and are not making a major contribution to familial breast cancer. Our data are consistent with epidemiological studies, which suggest that heterozygous FA mutation carriers are not at increased risk of breast cancer. Because we have screened only 88 BRCA1/2-negative breast cancer families it remains possible that some FA gene mutations do confer a high risk of breast cancer, although if this were the case they must be rare in comparison with BRCA1 or BRCA2 mutations. (By way of comparison, a family set of this type would be expected to contain ~20 deleterious BRCA1 or BRCA2 mutations). However, our current analyses do not exclude the possibility that FA gene sequence variants are associated with low penetrance breast cancer susceptibility similar to CHEK2 1100delC (21). Evaluation of this hypothesis will require additional studies comparing the frequency of FANC gene sequence variants in large series of breast cancer cases and healthy controls.

Acknowledgments

We thank all individuals and families who have participated in these studies. We thank Anita Hall and Karen Redman for family ascertainment, and Chris Mathew for positive controls for FANCA deletions.

References


Evaluation of Fanconi Anemia Genes in Familial Breast Cancer Predisposition

Sheila Seal, Rita Barfoot, Hiran Jayatilake, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/63/24/8596

Cited articles
This article cites 19 articles, 3 of which you can access for free at:
http://cancerres.aacrjournals.org/content/63/24/8596.full#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/63/24/8596.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.