Evaluation of Fanconi Anemia Genes in Familial Breast Cancer Predisposition


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 FA genes. Only 2 conservative missense variants, 1 in FANCA and 1 in FANCC, were identified of which 25 were exonic. None of the exonic variants resulted in translational frameshifts 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 FANCA 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, FANCE, FANC, and FANC. Thirty-five sequence variants were identified of which 25 were exonic. None of the exonic variants resulted in translational frameshifts 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 FANCA 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.

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Notes: The Breast Cancer Susceptibility Collaboration (UK) members that supplied families: Audrey Ardem-Jones, Rachel Belk, Nicola Bradshaw, Angela Brady, Barbara Bullman, Roseanne Cetnar, Cyril Chapman, Trevor Cole, Gillian Crawford, Carol Cummings, Rosemarie Davidson, Alan Donaldson, Diana Eccles, Rosalind Edles, Gareth Evans, Sheila Goff, Jonathon Gray, Helen Gregory, Neva Haite, Shirley Hodgson, Tessa Homfray, Richard Houlton, Louise Leat, Liane Jackson, Lisa Jeffers, Fiona Kelso, Mark Longmuir, Donna McIntyre, James Mackay, Alex Magee, Salma Mansour, Patrick Morrison, Vicky Murray, Joan Paterson, Mary Poutous, Nazneen Rahman, Mark Rogers, Andy Schofield, Sue Shanley, Janet Shean-Simmonds, and Lesley Snadden. Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org).

Requests for reprints: Michael R. Stratton, The Cancer Genome Project, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambs, CB10 1SA, United Kingdom. Phone: 44-1223-494-951; Fax: 44-1223-494-969; E-mail: mrs@sanger.ac.uk.
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, Fancd2, Fancc, Fance, Fance, 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, Fanf, 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, 8; 5 cases, 10; 4 cases, 14; 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, 11; 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 Fanca (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 H11417D) or were not reported previously (Fanca L1143V; Fancc V60I, Fancc E417L; Fancd2 T896M; Fance R365K, Fance A502T; and Fanf 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 Fancc 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

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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 FANCN P129P, were detected only in controls and were not present in the breast cancer samples. These variants were detected only in controls and were not present in the breast cancer samples.


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


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