Evaluation of Fanconi Anemia Genes in Familial Breast Cancer Predisposition

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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 FANCA, FANCC, FANCD2, FANCE, FANCJ, and FANCG. Sixty-nine sequence variants were identified of which 25 were exonic. None of the exonic variants resulted in translational frameshifts or nonsense codons and 14 were identified of which 25 were exonic. None of the exonic variants 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 FANCA, FANCC, FANCD2, FANCE, FANCJ, and FANCG. Sixty-nine 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, I 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, FA-E, FA-F, and FA-G. 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 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

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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 Cau-
casians 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 (10) and was negative. The residual probabilities of carry-
ing 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, Fance,
Fancf, and Fancg were screened for small intragenic mutations by con-
formation 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 compro-
mise screening other parts of Fancd2, although we were able to detect poly-
morphisms reported previously and novel variants without apparent dif-
ficulty. 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 simulta-
neously by PCR. The resulting products were electrophoresed on an ABI 3100
sequencer (ABI Perkin-Elmer) and analyzed with GENOTyper soft-
ware. 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,
FanccD2, Fance, FanceF, and Fancg. Each family was charac-
terized 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, 18; 4
cases, 28; and 3 cases, 30. The number of breast cancer cases diag-
nosed 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 associ-
ated 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 trans-
lational 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 H11017D) 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

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6 The Fanconi Anemia Mutation Database, internet address: http://www.rockerfeller.
edu/FAAnemia/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-penetrance 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 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.

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References

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