

kenazi Jewish and Lithuanian Catholic ancestry, a child with Fanconi anemia and a medulloblastoma was a BRCA2*6174delT/886delGT compound heterozygote. Two other kindreds each contained a Fanconi anemia-afflicted child who developed medulloblastoma; one child was of Latin American ancestry and a compound heterozygote for BRCA2*I2490T/530insA and the other was African American and a compound heterozygote for BRCA2*Q3066X/E1308X. Median age of the Fanconi anemia-afflicted children at brain tumor diagnosis was 3.5 years. The co-occurrence of brain tumors, Fanconi anemia, and breast cancer observed in one of these kindreds constitutes a new syndromic association. Individuals who carry a germline BRCA2 mutation and who plan to have children with a partner of Ashkenazi Jewish descent should consider undergoing genetic counseling. [J Natl Cancer Inst 2003; 95:1548-51]

Shared Genetic Susceptibility to Breast Cancer, Brain Tumors, and Fanconi Anemia

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Fanconi anemia is an inherited disease characterized by bone marrow failure, congenital malformations, and predisposition to cancer. The breast cancer susceptibility gene BRCA2 was recently found to be associated with Fanconi anemia complementation group D1 (FA-D1). We examined four kindreds afflicted with Fanconi anemia for the presence of germline BRCA2 mutations. One kindred, of Ashkenazi Jewish ancestry, had five members who were diagnosed with breast cancer and two cousins who were BRCA2*6174delT/C3069X compound heterozygotes and had Fanconi anemia and brain tumors. In another kindred of Ash-

Fanconi anemia is an autosomal recessive disease characterized by developmental anomalies, bone marrow failure, cellular sensitivity to DNA cross-linking agents, and increased incidence of both hematologic and solid tumors (1). Genes for seven Fanconi anemia complementation groups (i.e., FANCA, FANCC, FANCD1, FANCD2, FANCE, FANCF, and FANCG) have so far been identified. D'Andrea and colleagues (2)

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recently reported that germline mutations in the breast cancer susceptibility gene BRCA2 are associated with the FA-D1 complementation group. We addressed the clinical relevance of this finding by examining the occurrence of BRCA2 mutations among four kindreds with Fanconi anemia and pediatric brain tumors.

The kindreds described in this report were enrolled in the International Fanconi Anemia Registry (IFAR), a research study at The Rockefeller University (New York, NY). The study was approved by the Institutional Review Board of The Rockefeller University, and all subjects provided written informed consent. Medical and family histories were obtained by direct interview. The pedigree shown in Fig. 1 was modified to preserve the confidentiality of the family members. As part of the IFAR study (3,4), blood was collected from all family members, and genomic DNA was extracted for mutational analyses by

standard methods. Fibroblasts were isolated from skin biopsy samples obtained from selected patients and used for correction of cross-link hypersensitivity assays. As part of the IFAR study, linkage analysis was performed using microsatellite markers flanking the Fanconi anemia complementation group A locus (FANCA) on chromosome 16q24.3 (5). In selected kindreds (e.g., kindred 3), we excluded Fanconi anemia complementation groups FA-A, FA-C, FA-D2, FA-E, FA-F, and FA-G by examining the correction of cross-link hypersensitivity in patients' fibroblasts that had been transduced with retroviral vectors containing wild-type FANCA, FANCC, FANCD2, FANCE, FANCF, or FANCG complementary DNAs as previously described (6). Analysis of the coding regions and intron/exon junctions of BRCA2 was performed by direct DNA sequencing (Myriad Genetic Laboratories, Salt Lake City, UT) or allele-specific analysis of genomic DNA (7). Pathology reports or

medical record documentation of tissue diagnosis was available for all individuals diagnosed with Fanconi anemia except the proband in kindred 1, who underwent no autopsy.

In kindred 1, the proband (VI:4) was a male child of Ashkenazi Jewish ancestry who had multiple congenital anomalies visible at birth (Fig. 1, Table 1). Diepoxybutane testing performed on a blood sample obtained from this child at 16 months of age demonstrated increased chromosomal breakage, consistent with the clinical diagnosis of Fanconi anemia. At 4 years 10 months of age, the child had a computed tomography scan that revealed a large hyperdense mass in the midline of the posterior fossa. Subsequently, the child's health rapidly deteriorated and he died; an autopsy was not performed. A maternal grandaunt (IV:2), the paternal grandmother (III:10) of the proband, and a paternal grandaunt (i.e., sister of individual III:11) had breast cancer. The

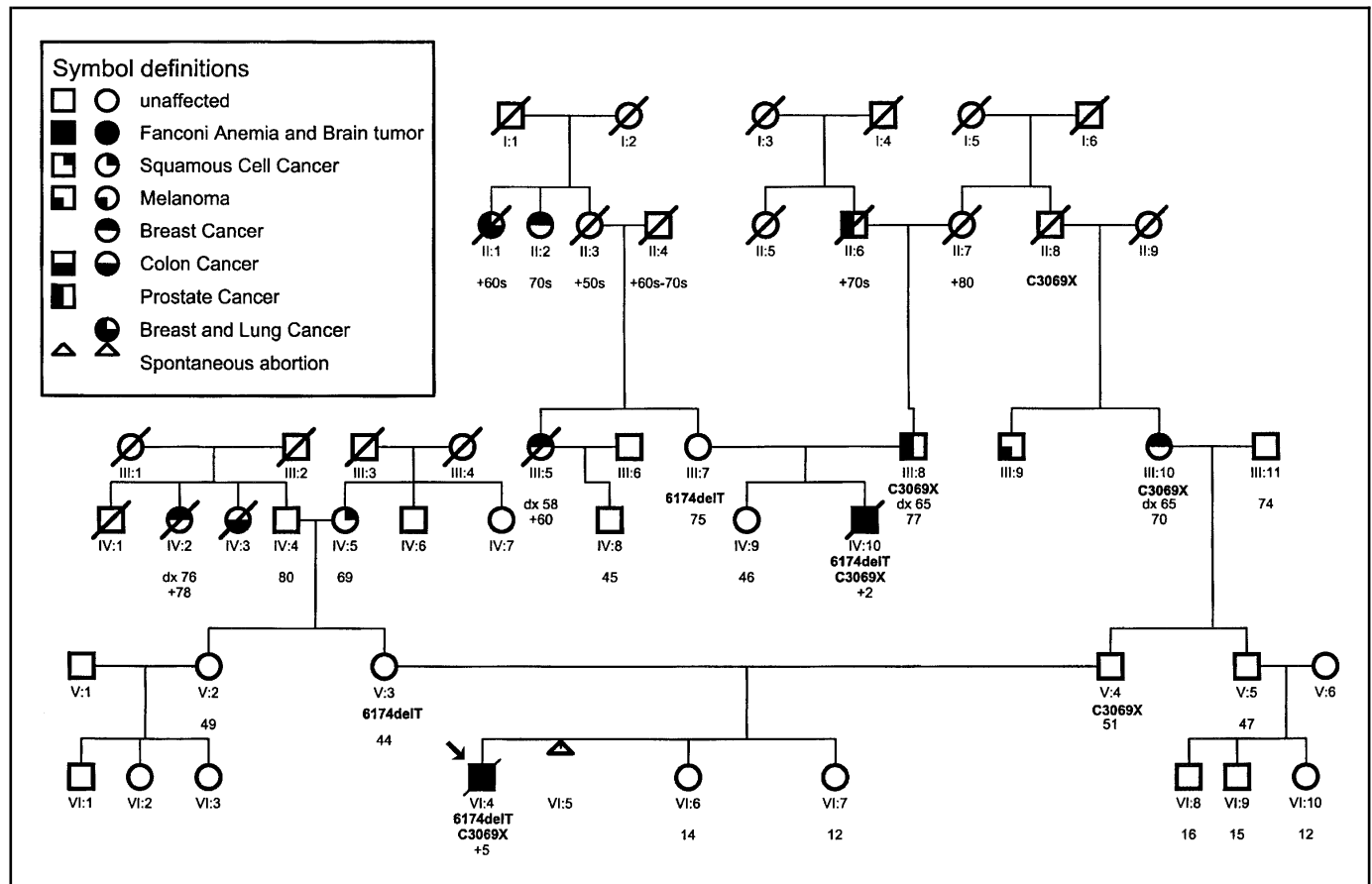


Fig. 1. Pedigree of kindred 1. The proband (arrow) was a child affected with Fanconi anemia. Numbers under the symbols refer to age at diagnosis if preceded by a "dx," age at death if preceded by a "+," and current age in years if the number has no preceding symbol. C3069X indicates that the patient carried the BRCA2**C3069X* mutation, and 6174delT indicates that the patient carried the BRCA2**6174delT* mutation. Diagnoses of cancer were confirmed

by review of a pathology report for individuals III:10 and IV:5 and by post-mortem examination for individual IV:10. No autopsy was performed to confirm radiologic diagnosis of the brain tumor in individual VI:4. Other cancer diagnoses were by family report. An additional individual (not shown on pedigree), the sister of individual III:11, also had breast cancer.

Table 1. Genotypes and phenotypes in kindreds co-segregating BRCA2 mutations

Kindred	Ancestry	Sex of the Fanconi anemia proband	Type of brain tumor	Age at brain tumor diagnosis, y	BRCA2 mutation 1 (exon)	BRCA2 mutation 2 (exon)
1	Ashkenazi Jewish	Male	Posterior fossa*	5	6174delT (11)	C3069X 9435T>A (24)
		Male	Astrocytoma	2	6174delT (11)	C3069X 9435T>A (24)
2	Ashkenazi Jewish/Lithuanian Catholic	Female	Medulloblastoma	4.5	6174delT (11)	886delGT (8)
3	Latin American	Female	Medulloblastoma	2.5	5301insA (11)	I2490T† 7690T>C (15)
4	African American	Female	Medulloblastoma	3.5	E1308X 4150G>T (10)	Q3066X 9424C>T (24)

*No autopsy was performed in this case; radiologic findings were consistent with a medulloblastoma or astrocytoma.

†Variant of unknown clinical significance was reported in the Breast Cancer Information Core database in more than 70 individuals of Latin American ancestry.

proband's second cousin once removed (IV:10) was also born with characteristics diagnostic of Fanconi anemia and died at 2 years of age; an autopsy revealed multiple astrocytomas in the child's cerebellum. Analysis of stored genomic lymphocyte DNA from the proband in kindred 1 failed to identify any mutation in FANCC, including the FANCC*IVS4+4A>T mutation that is prevalent among Ashkenazi Jews (3), suggesting that the members of this family were unlikely to be in that complementation group (Auerbach AD: unpublished data); single-strand conformation polymorphism analysis failed to detect any mutations at the FANCG locus (8). BRCA2 DNA sequence analysis of the proband revealed that he was a BRCA2*6174delT/C3069X compound heterozygote. The BRCA2*9435T>A mutation that was predicted to result in a premature stop codon at amino acid residue 3069 (i.e., C3069X) was also detected in five of the proband's paternal relatives (V:4, III:10, II:8, III:8, and IV:10) (Fig. 1). Individual III:7 (the wife of individual III:8), a 75-year-old woman with a family history of breast cancer, carried the BRCA2*6174delT allele. These latter two individuals were the parents of individual IV:10, the second child affected with Fanconi anemia in this kindred.

Kindred 2 was of mixed Ashkenazi Jewish and Lithuanian Catholic ancestry with no family history of breast cancer and included a girl with Fanconi anemia who was diagnosed with a medulloblastoma at age 4.5 years (Table 1). The proband in kindred 2 also carried the

BRCA2*6174delT mutation as well as the BRCA2*886delGT mutation.

Kindreds 3 and 4, which were of Latin American and African American ancestry, respectively, each included a girl with Fanconi anemia who developed medulloblastoma at age 2.5 years and 3.5 years, respectively. The members of kindred 3 were not in Fanconi anemia complementation groups FA-A, FA-C, FA-D2, FA-E, FA-F, or FA-G. The Fanconi anemia-affected children in kindreds 3 and 4 were compound heterozygotes for BRCA2*I2490T/5301insA and BRCA2*Q3066X/E1308X, respectively.

We have previously reported that the carrier frequency of the breast and ovarian cancer-predisposing mutation BRCA2*6174delT is approximately 1 in 100 among individuals of Ashkenazi Jewish heritage (9). We believe that the second mutation identified in kindred 1, BRCA2*9435T>A (C3069X), is also likely to be associated with an increased risk of breast cancer because another BRCA2 protein-truncating mutation located further downstream of C3069X, Y3308X, is associated with breast cancer (10). Two other protein-truncating alleles of BRCA2, 9900insA and 7691insAT, have been detected in a cell line derived from a Fanconi anemia patient in complementation group FA-D1 (2). However, in that report, family histories of breast and ovarian cancers of the patients from which that cell line and several other cell lines were derived are not known because medical records were not available (2).

It has been assumed that in humans,

as in mice, homozygous mutations of BRCA2 result in embryonic lethality (10). The phenotypes of human BRCA2 compound heterozygotes are less well defined. In four of the five cases of Fanconi anemia reported here, and in one compound heterozygote previously reported (2), the presence of one protein-truncating BRCA2 allele may allow for partial activity of BRCA2 and expression of the Fanconi anemia phenotype. The common polymorphism BRCA2*ter3326 results in a carboxyl-terminal protein truncation because of the presence of a premature stop codon at amino acid 3326 (11). The identification of this polymorphism in a cell line derived from a Fanconi anemia patient in complementation group FA-B who was a BRCA2 compound heterozygote (2) suggests a pathogenic role for this allele. We reviewed the results of BRCA1 and BRCA2 mutation tests conducted at a single reference laboratory in Salt Lake City, Utah, on more than 10000 members of breast cancer kindreds and found that 47 patients carried both the BRCA2*K3326X allele and another protein-truncating, and presumably disease-causing, BRCA2 mutation (supplemental data, available at <http://jncicancerspectrum.oupjournals.org/jnci/content/vol195/issue20/>). Because of the very low frequency at which this allele co-segregates with most of the other protein-truncating mutations observed, we predict that the majority of the dual mutation combinations observed occur in the *trans* configuration; none of the 47 patients had an unusual phenotype noted in the medical information pro-

vided by their physicians. The I2490T allele of BRCA2 that we detected in kindred 3 has been reported in more than 70 individuals of Latin American ancestry who were either unaffected or affected by breast cancer and is classified as a variant of unknown clinical significance in the Breast Cancer Information Core database (10). On the basis of these observations, we do not believe that the BRCA2*I2490T and BRCA2*K3326X alleles are likely to be pathogenic. We propose instead that an unidentified BRCA2 mutation or a mutation in another gene may have also been present in the kindreds with members who were compound heterozygotes for the BRCA2*I2490T (this report) or for the BRCA2*K3326X allele (2).

These findings provide the rationale for offering genetic counseling to individuals who carry a germline BRCA2 mutation and who plan to have children with a partner of Ashkenazi Jewish descent. Among Ashkenazi Jewish kindreds, numerous BRCA2 protein-truncating alleles, including BRCA2*9325insA, have been reported in the 3' region surrounding nucleotide 9435 (10,12,13). For example, the BRCA2*886delGT allele detected in the maternal lineage of kindred 2 has been observed 12 times in the Breast Cancer Information Core database (10). Thus, although the probability of an Ashkenazi Jewish carrier of the BRCA2*6174delT mutation having offspring with a carrier of another BRCA2 mutation is quite low, the possibility that such a couple could have offspring with Fanconi anemia has now been documented in this study. In addition, for individuals known to carry BRCA2 mutations, particularly mutations that result in a carboxyl-terminal protein truncation, the approximately 1% probability of the spouse (if of Ashkenazi Jewish descent) carrying a BRCA2*6174delT mutation (9) and the potential 25% risk of having an offspring with Fanconi anemia suggest that genetic counseling in this setting may be indicated.

Although there is no increased occurrence of brain cancers in kindreds with heterozygous BRCA2 mutations (14), both medulloblastomas and astrocytomas have been reported in rare cases of Fanconi anemia (1,4,15-17). The five cases of Fanconi anemia and brain tu-

mors analyzed for BRCA2 mutations in this study represent five of the six such cases reported to the IFAR. The finding of BRCA2 mutations in five children with Fanconi anemia and brain tumors, predominantly medulloblastomas, is distinctive and constitutes a new syndromic association. The close interactions between the FANCD1 and BRCA2 gene products and other cell-cycle checkpoint or DNA-damage response proteins, including ATM, BRCA1, NBS1, CHEK2, and RAD51, suggest possible mechanisms for the molecular pathogenesis of the brain tumors observed in these and possibly other kindreds (18).

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NOTES

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