Letters to the Editor

Am. J. Hum. Genet. 58:881–885, 1996

Germ-Line *BRCA1* Mutations in Selected Men with Prostate Cancer

To the Editor:

Prostate cancer is the most common malignancy among American men (American Cancer Society 1995, pp. 10–11). Although the causes of prostate cancer are unknown, a family history of the disease is one of the strongest risk factors identified, to date. Men with an affected first-degree relative with prostate cancer have at least a two-to-three-fold-higher risk of developing the disease compared with men who have no such family history (Carter et al. 1992; Whittemore et al. 1995).

Epidemiological observations also suggest a clustering of prostate and breast cancers in some families (Thiessen et al. 1974; Anderson et al. 1992, 1993; Tulinius et al. 1992; Sellers et al. 1994). More specifically, at least two studies suggest that inherited mutations in highly penetrant dominant genes such as *BRCA1,* which predisposes women to breast and ovarian cancers (Hall et al. 1990; Miki et al. 1994; Easton et al. 1995), may also be associated with an increased risk of prostate cancer in men. In a study of seven large Icelandic breast cancer families, two of which showed evidence of linkage to *BRCA1,* prostate cancer was found to be the second most frequent malignancy after breast cancer (Arason et al. 1993). Among presumed paternal carriers of mutant breast cancer gene alleles, 44% had a history of prostate cancer. Additional evidence regarding *BRCA1* and prostate cancer risk comes from an analysis of 33 *BRCA1*-linked families (Ford et al. 1994). This cooperative study estimated a relative risk of prostate cancer of 3.33 (95% confidence interval 1.8–6.2) among men carrying mutations, compared with the general population.

In order to assess directly the potential role of *BRCA1* mutations in the etiology of prostate cancer, we have screened for germ-line *BRCA1* mutations in a subset of men from an ongoing population-based case-control study of prostate cancer in men <65 years of age. Cancer cases were identified through the Cancer Surveillance System of northwestern Washington State, which is part of the Surveillance, Epidemiology, and End Results program, or SEER, of the National Cancer Institute. Eligible subjects (n = 344) were white or African American men newly diagnosed with histologically confirmed adenocarcinoma of the prostate between January 1, 1993, and June 30, 1994, and who resided in King County, Washington. A total of 280 (81%) of the eligible subjects completed the detailed in-person interview, during which information was collected regarding any family history of malignancies, by primary site, in first-and second-degree relatives.

The subgroup of cases selected for this analysis included men in whom genetic factors were most likely to be relevant. Specifically, subjects met at least one of the following criteria: (1) <53 years of age at diagnosis of prostate cancer (n = 32); (2) a family history of breast cancer in a first-degree female relative diagnosed at <51 years of age (n = 13); or (3) a family history of prostate cancer in two or more male relatives, with at least one relative diagnosed at <56 years of age (n = 4). Sixty-one men met one or more of these criteria, and blood samples were available from 49 (80.3%) of them. Characteristics of the interviewed men and the 49 who were genotyped are shown in table 1. These data demonstrate the highly selected nature of the genetic case group with respect to age at diagnosis and family history. In addition, a higher proportion of genotyped subjects had stage C (regional) disease at diagnosis compared with all interviewed cases.

In order to estimate the population frequency of each rare *BRCA1* allele, genotyping was performed on a series of male controls without a history of prostate can-

Table 1

Prostate Cancer Cases: Distribution of Selected Characteristics

Characteristic	Cases Included in Analysis (n = 49) No. (%)	Cases Interviewed (n = 280) No. (%)
Age (years):		
40–49	13 (26.6)	16 (5.7)
50–54	24 (49.0)	51 (18.2)
55–59	6 (12.2)	97 (34.7)
60–64	6 (12.2)	116 (41.4)
Race:		
Caucasian	48 (98.0)	263 (93.9)
African-American	1 (2.0)	17 (6.1)
Family history of prostate cancer:		
None	33 (67.4)	206 (73.6)
First-degree relative	11 (22.4)	50 (17.9)
Second-degree relative only	5 (10.2)	24 (8.5)
Family history of breast cancer:		
No first-degree relative	31 (63.3)	242 (86.4)
First-degree relative	18 (36.7)	38 (13.6)
Stage of disease[a]:		
A	4 (8.2)	19 (6.8)
B	18 (38.8)	149 (53.2)
C	20 (40.8)	86 (30.7)
D	2 (4.0)	11 (3.9)
Unknown	4 (8.2)	15 (5.4)

[a] According to the American/Whittemore staging system.

cer. These men were white or African American residents of King County, Washington, who were identified as controls through random digit dialing (Waksberg 1978; Harlow and Davis 1988) and were frequency matched to cases by age (same 5-year group). A total of 245 eligible controls with 1993 reference dates (which approximate the distribution of case diagnosis dates) were identified. At the time of this analysis, 176 (72%) controls had completed the detailed interview, which ascertained medical and family history, and a blood sample had been obtained for 145 (82%) of them. Controls genotyped for this study were similar to all interviewed controls according to age, race, and family history of prostate cancer or breast cancer (table 2).

Genomic DNA was prepared directly from peripheral blood, and *BRCA1* mutation screening was carried out by a combination of SSCP analysis and allele-specific assays. Forty-eight previously described primer pairs (Friedman et al. 1994; Friedman 1995) were used to amplify the BRCA1 coding sequence and promoter region for SSCP analysis. Five allele-specific assays were also performed using oligonucleotide probes for mutations in exons 2, 11, and 20 and splice-donor and -acceptor sites in intron 5 (Friedman 1995). All sequence variants were characterized by cycle sequencing of the PCR product by use of an ABI 373A sequencer.

The germ-line alterations detected in this study and characteristics of the seven prostate cancer subjects in whom they were found are shown in table 3. One previously described *BRCA1* mutation and five different rare sequence variants (one of which was detected in two unrelated men) were identified in this series of 49 cases. The functional significance of the alterations included in the latter category is not known, although none of these sequence changes was identified in the 145 population-based controls.

The one known mutation identified in this study is the frameshift mutation in exon 2 (subject 126), which produces a premature stop codon near the amino terminus of the *BRCA1* protein. This is the most common germ-line *BRCA1* mutation reported, to date (Shattuck-Eidens et al. 1995; Struewing et al. 1995*a*, 1995*b*; Tonin et al. 1995), and has been identified in the context of a shared haplotype in apparently unrelated families, suggesting a possible founder effect (Simard et al. 1994; Struewing et al. 1995*b*). Most of the families in which this mutation has been seen are of eastern European Jewish descent (Struewing et al. 1995*b*; Tonin et al. 1995), and subject 126 is Jewish. Subject 126 has no family history of breast or ovarian cancer; however, his father and two maternal relatives also carry a diagnosis of prostate cancer. Subject 126 has no sisters or maternal aunts, although his mother lived into her 70s before developing cancer of a nonreproductive site; he had paternal aunts who died without a history of cancer and has several children <40 years of age who also have no history of cancer.

Five rare sequence variants of unproved significance

Table 2

Population-Based Controls: Distribution of Selected Characteristics

Characteristic	Controls Included in Analysis (n = 145) No. (%)	Controls Interviewed (n = 176) No. (%)
Age (years):		
40–49	13 (9.0)	14 (8.0)
50–54	29 (20.0)	32 (18.2)
55–59	53 (36.5)	63 (35.8)
60–64	50 (34.5)	67 (38.1)
Race:		
Caucasian	141 (97.2)	171 (97.2)
African-American	4 (2.8)	5 (2.8)
Family history of prostate cancer:		
None	116 (80.0)	143 (81.2)
First-degree relative	16 (11.0)	20 (11.4)
Second-degree relative only	13 (9.0)	13 (7.4)
Family history of breast cancer:		
No first-degree relative	130 (89.7)	156 (88.6)
First-degree relative	15 (10.3)	20 (11.4)

Table 3

BRCA1 Sequence Alterations Identified in Men with Prostate Cancer

Alteration Type and Subject No.	Age (years) at Diagnosis[a]	Nucleotide Change	Consequence	Family History[a]
Known mutation:				
126	61–65	AG deletion at nt 185	Premature termination at amino acid 39	Father with prostate cancer at age 71–75 years Mother with nonreproductive-site cancer at age 71–75 years Two maternal second-degree relatives with prostate cancer at (1) age 46–50 years and (2) age unknown
Rare sequence variants:[b]				
89	46–50	12-bp insertion in intron 20	Unknown	Maternal second-degree relative with brain tumor at age 61–65 years
100	51–55	12-bp insertion in intron 20	Unknown	Mother with breast cancer at age 41–45 years
119	51–55	A to G at nt 3537	Ser 1140 Gly	Mother with breast cancer at age 36–40 years Two brothers with prostate cancer at ages 61–65 years Sister with genital tract cancer at age 56–60 years
86	46–50	C to T at nt 3	Noncoding exon	Father with fibrosarcoma at age 61–65 years Paternal second-degree relative with leukemia, age unknown
99	51–55	G to A at nt 58	Noncoding exon	Mother with malignancy of unknown primary site at age 81–85 years
108	51–55	C to T at nt 11 of intron 1	Noncoding	Father with colon cancer at age 51–55 years and lung cancer at age 66–70 years Paternal second-degree relative with brain tumor, age unknown

[a] Second-degree relatives are not identified, and ages of diagnosis of subjects and family members are presented as 5-year intervals, in order to maintain patient confidentiality.

[b] None of the alterations presented was identified in 145 population-based control individuals.

were identified in six cases from the study but not in the 145 population-based controls. The most provocative of these is a 12-bp insertion in intron 20, which was identified in two cases (subjects 89 and 100). This alteration is located 48 bp downstream of the 3′ end of exon 20 and represents a reduplication of the immediately adjacent sequence. This alteration has previously been found in the germ line of a woman diagnosed with cervical cancer at age 26 years and breast cancer at age 31 years (Langston et al. 1996), and it has also been described in a woman with a history of both breast and ovarian cancer, who has five maternal relatives with breast cancer (Takahashi et al. 1995). This insertion may affect RNA processing; however, cDNA from these individuals is not available for experiments to provide formal proof of such an effect.

Four rare sequence variants were identified in both coding and noncoding regions of the *BRCA1* gene. The single-base-pair substitution in exon 11 (subject 119), which produces a Ser-to-Gly amino acid change, has not been described in studies of either families or tumor specimens, and further studies will be required to assess its functional significance. Three rare variants in noncoding regions were also found, two within the 5′-UTR in exon 1 (subjects 86 and 99) and one in intron 1 (subject 108). None of these three sequence variants have been reported previously. One additional point mutation at nt 49 of exon 4, which is a noncoding exon, was identified in two study subjects, as well as in five controls, and is unlikely to be of functional importance.

Of the 49 cases genotyped, 7 (100%) of 7 of those with germ-line alterations and 39 (93%) of the 42 without evidence of alterations reported a positive family history of cancer in first-and/or second-degree relatives. Similar proportions of individuals in each group reported first-degree relatives affected with prostate cancer or breast cancer.

In this preliminary study of selected men with prostate cancer, we have identified one germ-line *BRCA1* mutation, as well as five rare sequence variants, that were not identified in the population-based control group. A recent genetic epidemiological analysis estimates the population frequency of *BRCA1* mutations to be ~.0006 (Ford et al. 1995). Another study suggests that the frequency of the 185delAG mutation may be as high as 1% in Ashkenazi Jewish populations (Struewing et

al. 1995*a*). Taken in this context, the predicted importance of *BRCA1* in prostate cancer hinges on the significance of the five rare variants identified. The fact that none of the sequence variants was identified in controls suggests that one or more of these may ultimately be shown to represent alleles predisposing to disease. The expressivity and penetrance of these alleles may be substantially different from the patterns associated with alleles identified in high-risk breast/ovarian cancer families.

A recent epidemiological analysis failed to identify a significantly increased risk of breast cancer among relatives of prostate cancer probands (Isaacs et al. 1995). Our findings are not necessarily in conflict with those of Isaacs et al., since the contribution of germ-line *BRCA1* mutations to the overall incidence of prostate cancer appears to be small, at most, and may be limited to specific subgroups of patients. Larger population-based studies of both tumor and normal tissue from men with prostate cancer are warranted to more precisely define the role of both germ-line and somatic mutations in *BRCA1* in prostate cancer development.

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Acknowledgments

We thank Leigh Francisco for technical assistance with DNA sequencing. In addition, we are grateful to the many men who participated in this study. This work was supported in part by National Cancer Institute grant R01 CA56678 (to J.L.S.) and contract N01-CN-05230; awards from the CaP Cure Foundation (to J.L.S. and E.A.O.); and institutional funds from the Fred Hutchinson Cancer Research Center. A.A.L. is the recipient of a Physician Scientist Award K11 HD00936 from the National Institutes of Health. E.A.O. is the recipient of an American Cancer Society Junior Faculty Award JFRA-558.

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0002-9297/96/5804-0026$02.00

Am. J. Hum. Genet. 58:885–888, 1996

The Gene for Nijmegen Breakage Syndrome (V2) Is Not Located on Chromosome 11

To the Editor:

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by oculocutaneous telangiectasia and cerebellar ataxia. Individuals with this disorder display immunological impairments, hypersensitivity to ionizing radiation, and a predisposition to cancer (for reviews, see Shiloh 1995). There has been reported genetic heterogeneity in AT, which appeared to include four genetic complementation groups in classical AT—i.e., A, B/C, D, E—and two variants, so-called Nijmegen breakage syndrome (NBS), V1 and V2 (Murnane and Painter 1982; Jaspers et al. 1988). Among the four groups of classical AT, no significant differences in clinical appearance have been seen. Familial linkage analyses have produced evidence that genes for all four complementation groups in classical AT reside in a narrow region on chromosome 11q22-23 (Gatti et al. 1988; McConville et al. 1994). On the other hand, NBS patients have neither cerebellar ataxia nor telangiectasia but do display microcephaly and a developmental delay (Weemaes et al. 1981; Wegner et al. 1988). However, patients share features with AT, such as high radiosensitivity, radioresistant DNA synthesis (RDS), and chromosome instability (Jaspers et al. 1988), suggesting that the same pathway (or part thereof) is impaired in both syndromes. The underlying gene for NBS has not yet been identified, and its location in the human genome is still unknown.

The genetic complementation groups were defined by somatic cell fusion between AT cells from different AT patients: if the fused cells retained RDS, then the two patients belonged to the same groups. Each genetic complementation group within classical AT as well as NBS were believed to arise from different mutated genes or at least from different mutations within one or several genes. However, the recently cloned ATM gene was found to be mutated in all complementation groups of AT, without any correlation to whatever the complementation group, indicating that, at least in classical AT, only one single gene is involved (Savitsky et al. 1995). Whether the same ATM gene is also involved in NBS remains, as yet, unclear, since the complementation studies based on the restoration of RDS obviously give conflicting results (Komatsu st al. 1989; Verhaegh et al. 1993, 1995; Zdzienicka et al. 1994; Savitsky et al. 1995).

To further study the genetic complementation of NBS and AT on the basis of high-radiation sensitivity of both diseases, we transformed several primary cultures of NBS skin fibroblasts with SV40 virus, and an immortal cell line was successfully established only from GM7166 cells belonging to AT-V2. The passage number of these GM7166VA7 cells is presently 80, and the life span was sufficiently long to allow them to be used for subcloning of somatic-cell and microcell hybrid. In addition, the radiation sensitivity of GM7166VA7 cells was nearly the same as that of the parental primary cultures.

Using the GM7166VA7 cell line, we examined the genetic heterogeneity of NBS V2 and classical AT by an introduction of chromosome via microcell-mediated chromosome transfer. A single copy of human chromosome 11 was introduced into GM7166VA7 cells, and the karyotypes of the resulting G418-resistant clones were analyzed. Figure 1 shows metaphase chromosomes of GM7166VA7 cells and the microcell hybrid clone 11/1. GM7166VA7 cells contained two chromosomes 11, whereas the microcell hybrid clone 11/1 revealed a trisomy of chromosome 11 in 80% of the cells and a disomy in the remaining 20%. This disomy probably was the result of loss of either of the original parental chromosomes 11 or of the introduced chromosome 11, as has been previously observed in chromosome transfers to AT5BIVA cells (Komatsu et al. 1990). In order to confirm the presence of chromosome 11 derived from A9(neo11)-1 in the microcell hybrid clone, CA-repeat polymorphisms at the D11S420 locus on human chromosome 11q23.3-24 were analyzed in both GM7166VA7 recipient cells and A9(neo11)-1 donor