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Two Percent of Men with Early-Onset Prostate Cancer Harbor Germline Mutations in the *BRCA2* Gene

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Studies of families with breast cancer have indicated that male carriers of *BRCA2* mutations are at increased risk of prostate cancer, particularly at an early age. To evaluate the contribution of *BRCA2* mutations to early-onset prostate cancer, we screened the complete coding sequence of *BRCA2* for germline mutations, in 263 men with diagnoses of prostate cancer who were ⩽55 years of age. Protein-truncating mutations were found in six men (2.3%; 95% confidence interval 0.8%–5.0%), and all of these mutations were clustered outside the ovarian-cancer cluster region. The relative risk of developing prostate cancer by age 56 years from a deleterious germline *BRCA2* mutation was 23-fold. Four of the patients with mutations did not have a family history of breast or ovarian cancer. Twenty-two variants of uncertain significance were also identified. These results confirm that *BRCA2* is a high-risk prostate-cancer–susceptibility gene and have potential implications for the management of early-onset prostate cancer, in both patients and their relatives.

Introduction

Many studies have demonstrated that prostate cancer (PRCA) exhibits significant familial aggregation, particularly at a young age (reviewed by Eeles et al. 1999). Segregation analyses have suggested that this familial clustering is consistent with the existence of high-penetrance prostate-cancer–susceptibility alleles. Despite this, linkage searches in families with multiple cases of PRCA have been inconclusive. Loci on chromosomes 1q24, 1q42, 1p36, Xq27, and 17p11 have been mapped (Smith et al. 1996; Berthon et al. 1998; Xu et al. 1998; Gibbs et al. 1999), and the specific candidate genes *HPC1* (Carpten et al. 2002 [*RNASEL*; MIM 601518 and MIM 180435]) and *HPC2* (Tavtigian et al. 2001 [MIM 605367]) have been identified as high-risk PRCA genes, but none has thus far been definitively confirmed.

One other gene that has been implicated in PRCA predisposition is *BRCA2* (MIM 600185). The association with this gene was first suggested by studies in Iceland: Arason et al. (1993) found an excess risk of PRCA in families with multiple cases of breast cancer in Iceland, the majority of which have subsequently been shown to segregate a single *BRCA2* mutation, 999del5 (Thorlacius et al. 1996). Johannesdottir et al. (1996) found that 2.7% of patients with PRCA who were aged <65 years carried this mutation, compared with ~0.5% of the general population. Sigurdsson et al. (1997) estimated a PRCA relative risk of 4.6 in male first-degree relatives of patients with breast cancer in families segregating the 999del5 mutation. This association has been supported by a large collaborative study from the Breast Cancer Linkage Consortium (BCLC 1999). This study, based on 173 families harboring *BRCA2* mutations, estimated a relative risk of 4.65 (95% CI 3.48–6.22) for PRCA in male *BRCA2* gene carriers. The estimated relative risk rose to 7.33

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in patients diagnosed before the age of 65 years. In contrast, studies based on the Ashkenazi Jewish founder mutation 6174delT have not found a strong association between this mutation and PRCA risk (Struewing et al. 1997; Lehrer et al. 1998; Hubert et al. 1999; Nastiuk et al. 1999; Wilkens et al. 1999; Vazina et al. 2000). There is consistent evidence for loss of heterozygosity of the *BRCA2* region in PRCAs, particularly those at an advanced stage (Cooney et al. 1996; Melamed et al. 1997; Edwards et al. 1998; Watanabe et al. 1998; Hyytinen et al. 1999).

In a previous study (Gayther et al. 2000), we screened for *BRCA2* mutations in 38 patients with PRCA who had a family history of the disease. Deleterious mutations were found in 2 of the 16 patients who received diagnoses before the age of 60 years (at ages 52 and 56 years), whereas no mutations were found among the 22 older patients (who received diagnoses at or after the age of 60 years), providing further evidence of an increased risk of PRCA at young ages in *BRCA2* mutation carriers. To establish the contribution of *BRCA2* mutations to early-onset PRCA, we have now screened a total of 263 men diagnosed at ages $\leqslant$55 years, who were unselected for family history.

Material and Methods

Patients

Patients were recruited through the Cancer Research UK/British Prostate Group (formerly "CRC/BPG") UK Familial Prostate Cancer Study and the British Association of Urological Surgeons Section of Oncology, over the period 1992–1999. Three hundred and twelve patients who received diagnoses before the age of 56 years were identified through records of participating clinicians. This represents ~50% of patients who received diagnoses in the United Kingdom over the corresponding period. Patients were enrolled regardless of family history. Seven (2.2%) of the patients invited to participate had died before being approached, 4 (1.3%) declined to participate, and a further 37 (11.9%) did not provide a questionnaire or blood sample, leaving 264 patients to be analyzed.

All but one of the patients in the study had disease that was classified as adenocarcinoma of the prostate. The one exception was the youngest patient in the study, diagnosed at age 24 years with a low grade sarcoma. This patient was excluded from these analyses. The remaining 263 patients received diagnoses between the ages of 32 and 55 years (mean 51 years). Eleven (4%) of the patients from this group were of black African or Caribbean descent, and the remainder were white. Details of prostate and other cancers in first-degree relatives of the patients were obtained by questionnaire.

DNA Isolation

Lymphocytes were collected from patients and were stored in EDTA at −70°C until required. Lymphocyte DNA was extracted by routine methods (Edwards et al. 1997).

PCR

PCR and thermocycling was conducted as described by Edwards et al. (2001). Forty-five *BRCA2* primer pairs (M. Stratton and S. Gayther, personal communication) were used. The longer exons were amplified as subfragments and were numbered accordingly; there are four fragments for exon 10 (10.01–10.04), 17 fragments for exon 11 (11.01–11.17), and two fragments each for exons 14 and 27. Exons 5/6 and 23/24 were amplified in the same fragment. Primer sequences and PCR conditions are available from the authors' Web site.

Mutation Screening

Samples were analyzed by fluorescent mutation detection (F-MD) as described by Edwards et al. (2001). PCR products were mixed robotically (as many as 12 fragments per sample), which allowed the entire *BRCA2* gene of an individual to be screened in four lanes of an ABI 377. Heteroduplexes were identified by the presence of more than one peak (band shift) for a specific PCR product. Where band shifts were found, the mixed and unmixed PCR products were rerun on F-MD for confirmation. When PCR fragments were too weak or contained nonspecific amplimers, they were not placed in a post-PCR multiplex mix; these fragments were run on F-MD separately.

Sequencing

After confirmation of a band shift, the sample was reamplified by PCR from stock DNA, and sequencing was conducted to characterize mutations through use of an ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit and an ABI377 Genetic Analyzer, with both forward and reverse PCR primers.

Nomenclature

Sequence variants were categorized as either "deleterious," "polymorphisms," or "variants of uncertain significance" (VUS). Since no amino-acid substitutions in *BRCA2* have been shown to be clearly disease associated, the category of deleterious mutations was restricted to those that result in a truncated protein (frameshift insertion or deletion, nonsense mutation, or known pathogenic splice-site alteration). Polymorphisms were those with reported population frequencies >1%. All other missense variants in the coding and noncoding

regions were categorized as VUS (Frank et al. 2002; Breast Cancer Information Core [BIC] Web site).

Statistical Methods

Confidence intervals for the prevalence of *BRCA2* mutations among patients with PRCA were computed under the assumption that (given that the prevalence was relatively small) the number of mutations followed a Poisson distribution and, hence, that the variance of the log (prevalence) was given by the reciprocal of the number of observed mutations. Estimates of *BRCA2* prevalence in the general population were derived from two previous studies (Peto et al. 1999; Antoniou et al. 2002). Confidence limits for the prevalence estimate in the Peto et al. (1999) article were derived using a similar Poisson assumption based on the observed number of mutations; confidence limits for the estimate in the Antoniou et al. (2002) study are given in that article. These estimates were combined into a single inverse variance-weighted estimate. Confidence limits for the relative risk of PRCA in *BRCA2* mutation carriers were computed by assuming that the variance of the log (relative risk) was the sum of the variances of the log (prevalence) in patients with PRCA and the log (prevalence) in the general population.

Results

Mutation Detection (F-MD) and Sequencing

Examples of band shifts seen by F-MD are shown in figure 1. Five frameshift mutations were identified, in patients aged 47, 48, 52, 52, and 53 years at PRCA diagnosis (table 1). In addition, we found one splice-site mutation (IVS17-1g→c), in a 44-year-old patient. This mutation would be expected to result in skipping of exon 18 and has been reported six times previously as a splice-site mutation, on the BIC Web site. We have therefore classified this as a deleterious mutation. The sequence electropherograms of these mutations are shown in figure 2. All of the truncating mutations were found in white patients. Twenty-two variants of uncertain significance (VUS) in 24 patients (table 2) and 26 polymorphisms (table 3) were also identified.

Family History of Cancer

Of the six patients with protein-truncating mutations, one had a family history of PRCA. Two (PRY 086 and PRY 012) had a family history of breast/ovarian cancer (fig. 3). The sister of PRY086 was diagnosed with ovarian cancer, and four of his female relatives were diagnosed with breast cancer (three before age 60 years). A paternal aunt of PRY012 received a breast cancer diagnosis at age 68 years. Three of the mutation carriers had no family history of breast or ovarian cancer or PRCA.

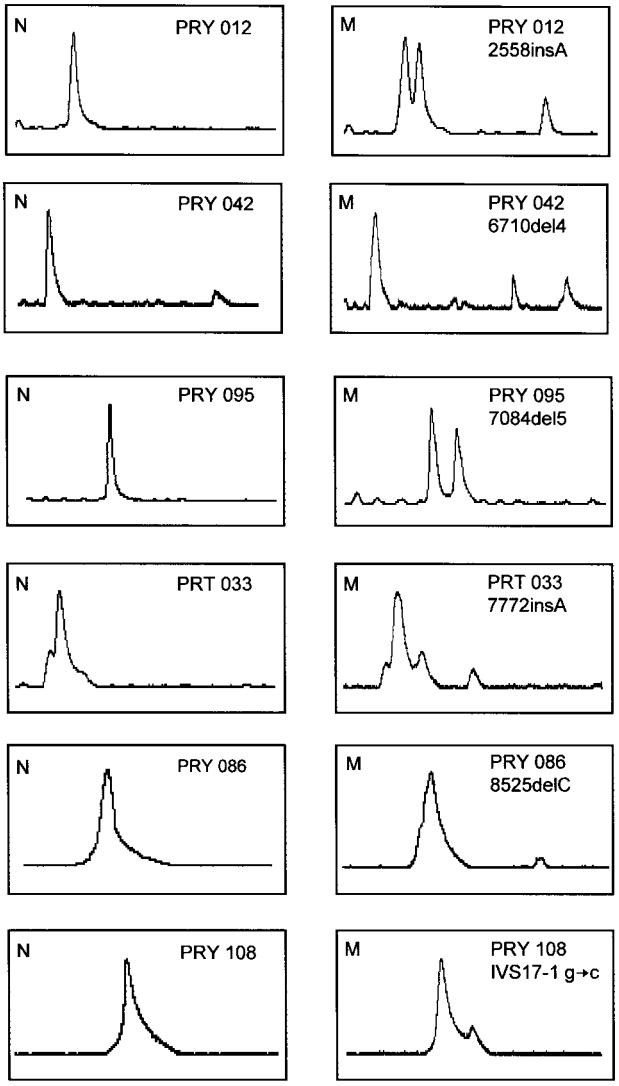


Figure 1 F-MD traces of deleterious alterations. Heteroduplexes are seen as band shifts from normal (N) and mutant (M) amplimers on F-MD gels. When band shifts were detected, samples were repeated at least once.

Discussion

We have analyzed the entire coding region of the *BRCA2* gene in a total of 263 patients with PRCA who received diagnoses at or before the age of 55 years, and we have found six protein truncating mutations (2.3%; 95% CI 0.8%–5.0%). This is likely to be an underestimate of the true frequency, since, like other mutation screening methods, our method will not have been able to detect large deletions or rearrangements, and some of the mis-

Table 1

Deleterious Alterations Found in 263 Patients with Early-Onset Prostate Cancer

Patient ID	Exon/ Intron (I)[a]	Age at Diagnosis (years)	Nucleotide Change	Protein Effect	BIC	Family History (Relationship, Age [in Years] at Diagnosis)		
						Prostate Cancer[b]	Breast or Ovarian Cancer	Other Cancer
PRY 012	11.02	48	2558insA	Stop 782	Yes	No	Breast (aunt, 68)	Larynx (father, 57; grandfather), cervix (aunt), lung (uncle; uncle, 56), liver (grandmother, 73)
PRY 042/201[c]	11.15	52	6710delACAA	Stop 2167	Yes	Brother, 48	No	Pancreas (grandmother, 55)
PRY 095	12	47	7084delAAAAG	Stop 2291	No	No	No	No
PRT 033	15	53	7772insA	Stop 2536	No	No	No	No
PRY 086[d]	18	52	8525delC	Stop 2776	Yes	No	Breast (grandmother; mother, 58; aunt, 58; cousin, 38); ovarian (sister, 46)	Lung (uncle, 59)
PRY 108	I17	44	IVS17-1g→c	Splicing error	Yes	No	No	No

[a] The longer exons were amplified as subfragments and were numbered accordingly; see the "Material and Methods" section for explanation. Intronic (I) sequences were present at the beginning and ends of shorter coding sequences.

[b] In first- and second-degree relatives.

[c] This mutation was also found by Gayther et al. (2000).

[d] PRY 086 also had another missense mutation 3′ to the deletion 8365A→G (T2713A).

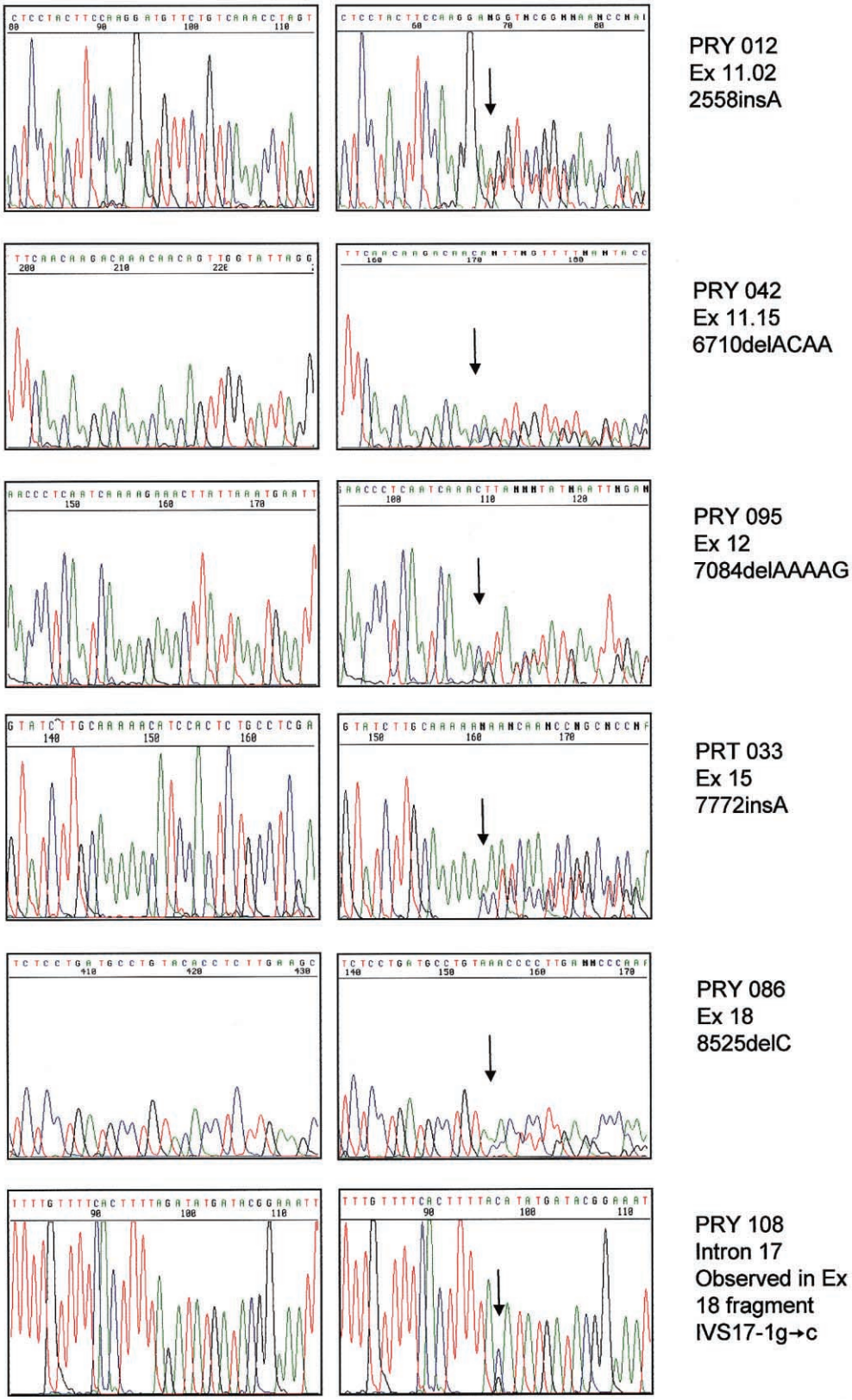


Figure 2 Electropherograms of mutations. *Left column*, normal sequence. *Right column*, mutant sequence. The arrow (↓) shows the position of the mutation.

Table 2

***BRCA2* VUS Found in Patients with Early-Onset Prostate Cancer**

Exon/Intron (I)[a]	Amino Acid Change	Nucleotide Change	Protein Domain (Where Known)[b]	Conservation Comparison with Mouse[c] (%)	Description on BIC[d]	No. Found in the Present Study	Family History (Type or No. Recorded, Age [in Years] at Diagnosis)		
							Prostate Cancer	Breast Cancer	Other Cancer
3	Y42C	353A→G	Transcriptional activation	100	UV (37)	1	No	No	No
10.01	S326R	1206C→A	P/CAF interaction, HAT activity	>50	UV (7)	1	No	No	No
10.03	I505T	1742T→C		100	UV (21)	1	No	No	No
11.01	Q713L	2366A→T		<50	UV (1)	1	No	No	No
11.03	D935H	3031G→C		100	…	1	No	1 (50)	No
11.03	D935N	3031G→A		100	UV (20)	1	No	No	Esophagus
11.04	L1019V	3283C→G	BRC repeats, Rad51 interaction	100	UV (5)	1	No	No	No
11.06	I1275M	4053A→G	BRC repeats, Rad51 interaction	100	…	1	No	No	Liver, uterus
11.07	L1457F	4599G→C	BRC repeats, Rad51 interaction	100	…	1	No	No	Liver, uterus
11.11	N1730Y	5416A→T	BRC repeats	100	…	1	No	No	Pancreas, lung
11.11	G1771D	5540G→A		>50	UV (6)	1	1 (86)	1 (44)	No
11.12	S1871N	5840G→A	BRC repeats	0	UV (1)	1	No	No	No
11.13	D2005V	6242A→T	BRC repeats, Rad51 interaction	>50	…	1	No	No	No
11.16	Y2222C	6893A→G		100	…	1	No	No	No
18	D2665G	8222A→G		100	UV (8)	1	No	1 (63)	Leukemia (12)
18	A2717S	8377G→T		100	UV (19)	2	No	No	Brain (77)[e]
20	E2856A	8795A→C		100	UV (29)	1	No	No	Colon, unkown
22	K2950N	9078G→T		100	UV (15)	1	3 (46, 57, 77)	No	No
25	Y3098H	9520T→C		<50	UV (6)	1	No	No	Kidney (34)
2	5′UTR A→C	214A→C		…	…	1	1 (70)	No	No
I6	IVS6-19c→t	intron 6-19c→t		…	UV (5), S (6)	2	No	No	No
I17	IVS17-1g→c	intron 17-1g→c		Splice site	S (6 Myr)	1	No	No	No

[a] The longer exons were amplified as subfragments and were numbered accordingly; see the "Material and Methods" section for explanation. Intronic (I) sequences were present at the beginning and ends of shorter coding sequences.

[b] P/CAF (transcriptional coactivator protein), HAT (histone acetylase transferase), BRC (30 amino acid repeats)

[c] Amino acid conservation was scored by the BESTFIT program (Wisconsin Computing Group) as 100% identical, >50% (indicates amino acid pairs having matrix scores >0.5), <50% (indicates positive scores <0.5%), or 0% (no similarity) (Connor et al. 1997).

[d] Values in parentheses represent the number of times there is an entry for the given mutation in the BIC database. UV = unclassified variant equivalent to VUS - variants of unknown significance, S = splice site, Myr = Myriad Genetics.

[e] Two A2717S mutations were detected separately in two patients; one of these patients received a diagnosis of brain cancer at age 77 years.

Table 3

***BRCA2* Polymorphisms Found in Patients with Early-Onset Prostate Cancer**

Exon/Intron (I)[a]	Amino Acid Change	Nucleotide Change	Protein Domain (Where Known)	BIC Classification (No. Recorded); % Global Heterozygosity[b]
2	5′UTRG→A	203G→A		P (9); 26
10.01	N289H	1093A→C		P (7), UV (1); 12
10.02	N372H	1342A→C	P/CAF interaction	P (7); 42
10.02	G425G	1503A→G	P/CAF interaction	No
10.02	S455S	1593A→G	P/CAF interaction	P (6); 11
11.01	G637G	2139T→C		No
11.01	H743H	2457T→C		P (2); 13
11.03	Q961Q	3111G→A		P (1), UV (1); 0.6
11.03	N991D	3199A→G	BRC repeats, Rad51 interaction	P (2); 15
11.04	K1132K	3624A→G		P (6); 34
11.04	P1088P	3492T→C		No
11.05	V1269V	4035T→C		P (3); 23
11.06	L1356L	4296G→A		P (1)
11.07	D1420Y	4486G→T	BRC repeats, Rad51 interaction	P, UV (103); 3
11.09	Q1562Q	4914A→G	BRC repeats, Rad51 interaction	No
11.09	D1575D	4953C→T	BRC repeats, Rad51 interaction	No
11.12	T1915M	5972C→T		P (1), UV (4); 5
11.13	R2034C	6328C→T	BRC repeats, Rad51 interaction	UV (65); 0.6[c]
14.01	S2414S	7470A→G		P (8), UV (1); 25
19	V2820V	8688A→C		No
22	A2951T	9079G→A		P (33), UV (5); 0.6
27.02	K3326X	10204A→T		P (2), UV (154); 1.2
27.02	R3370R	10338G→A		P, UV (2)
27.02	I3412V	10462A→G		P (1), UV (81); 2.4
I8	IVS8+56c→t	909+56c→t		P (1); 2.4
I24	IVS24-16t→c	I24-16t→c		P (1), UV (3)

[a] The longer exons were amplified as subfragments and were numbered accordingly; see the "Material and Methods" section for explanation. Intronic (I) sequences were present at the beginning and ends of shorter coding sequences.

[b] Data on global heterozygosity were obtained from Wagner et al. (1999). UV = unclassified variant equivalent to VUS, P = polymorphism.

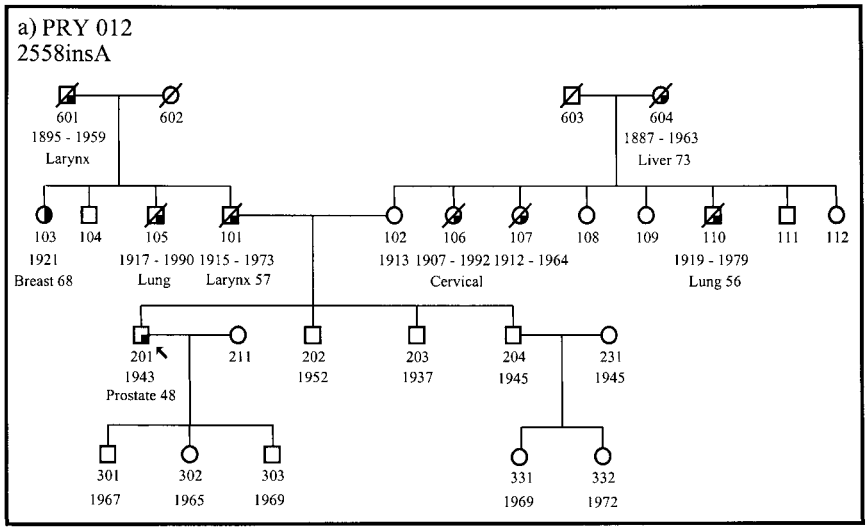
[c] Listed as "UV" on BIC; however, we consider this to be a polymorphism. Wagner et al. (1999) also state this.

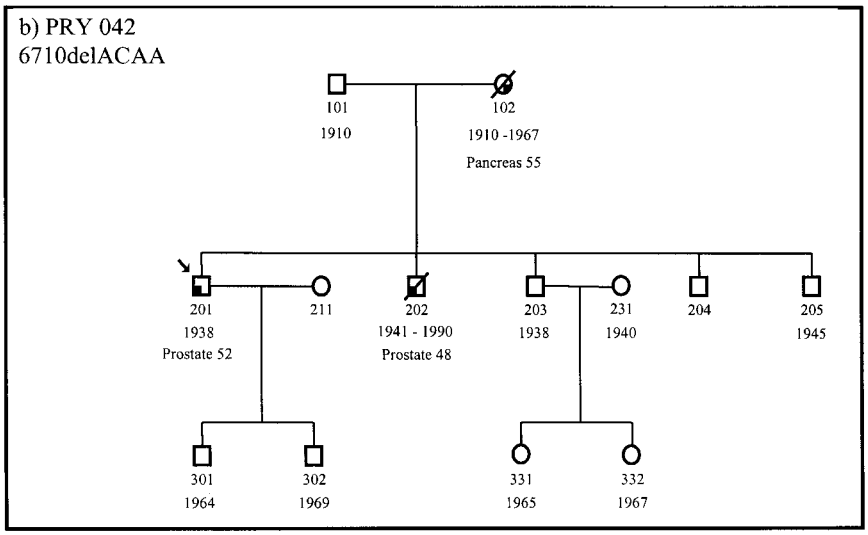
sense variants may be disease causing. The BCLC has estimated that 78% of families that show linkage to *BRCA2* have demonstrable truncating mutations in the gene (D. Easton, personal communication). On the basis of this sensitivity, we estimate the overall prevalence of *BRCA2* mutations in early-onset PRCA to be 2.9% (95% CI 1.0%–6.4%).

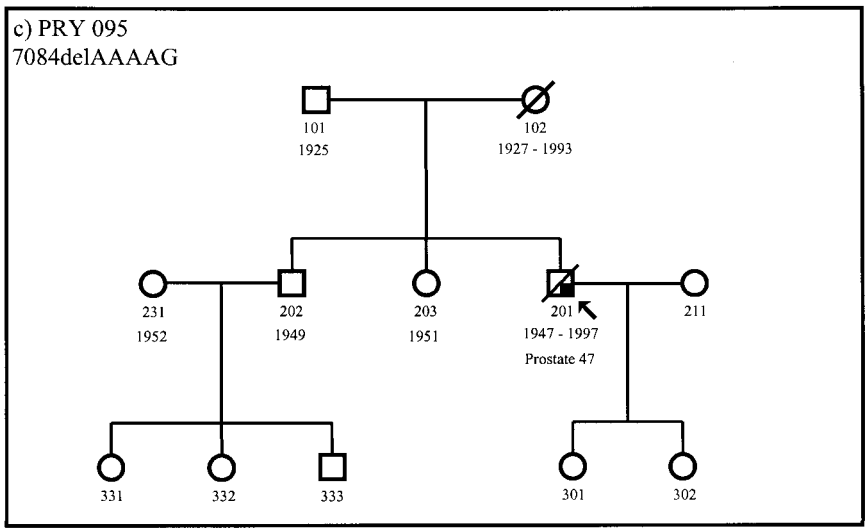
Patients in this series were selected solely on the basis of age at diagnosis, without regard to family history or disease severity. Among patients identified by participating clinicians, >80% of patients were included, and only seven had died before they could be included. Thus, although some selection bias cannot be ruled out, this series should be reasonably representative of early-onset PRCA in general.

Comparison of our prevalence estimate with the prevalence of *BRCA2* mutations in the general population can provide an estimate of the relative risk of early-onset PRCA in carriers. The frequency of *BRCA2* mutations in the general U.K. population has not been estimated directly but has been estimated indirectly from the frequency of mutations in patients with early-onset breast cancer. In separate studies, Antoniou et al. (2002) estimated a carrier frequency of 0.14% (95% CI 0.07%–0.28%) and Peto et al. (1999) estimated a frequency of 0.12% (0.07%–0.20%). On the basis of the average of these two estimates, the relative risk of early-onset PRCA is ~23-fold (95% CI 9–57). This is significantly higher than the 7.3-fold estimated from the BCLC study, for patients diagnosed before age 65 years ($P = .025$), consistent with an increasing trend in relative risk with early age at diagnosis (although both estimates have wide confidence limits). The estimated cumulative risks of PRCA by age 55 and 65 years in the general population are ~0.06% and 1.50%, respectively, on the basis of recent England and Wales cancer registration rates (Parkin et al. 1992). We therefore estimate the absolute risk of PRCA in *BRCA2* carriers to be ~1.3% by age 55 years and 10% by age 65 years.

Thompson et al. (2001) found that the risk of PRCA was lower in carriers of *BRCA2* mutations in the ovarian cancer cluster region (OCCR; nucleotides 3035–6629) than in carriers of other mutations (relative risk


a) PRY 012
2558insA
601
1895 - 1959
Larynx
602
603
604
1887 - 1963
Liver 73
103
1921
Breast 68
104
105
1917 - 1990
Lung
101
1915 - 1973
Larynx 57
102
1913
106
1907 - 1992
107
1912 - 1964
Cervical
108
109
110
1919 - 1979
Lung 56
111
112
201
1943
Prostate 48
211
202
1952
203
1937
204
1945
231
1945
301
1967
302
1965
303
1969
331
1969
332
1972


b) PRY 042
6710delACAA
101
1910
102
1910 -1967
Pancreas 55
201
1938
Prostate 52
211
202
1941 - 1990
Prostate 48
203
1938
231
1940
204
205
1945
301
1964
302
1969
331
1965
332
1967


c) PRY 095
7084delAAAAG
101
1925
102
1927 - 1993
231
1952
202
1949
203
1951
201
1947 - 1997
Prostate 47
211
331
332
333
301
302

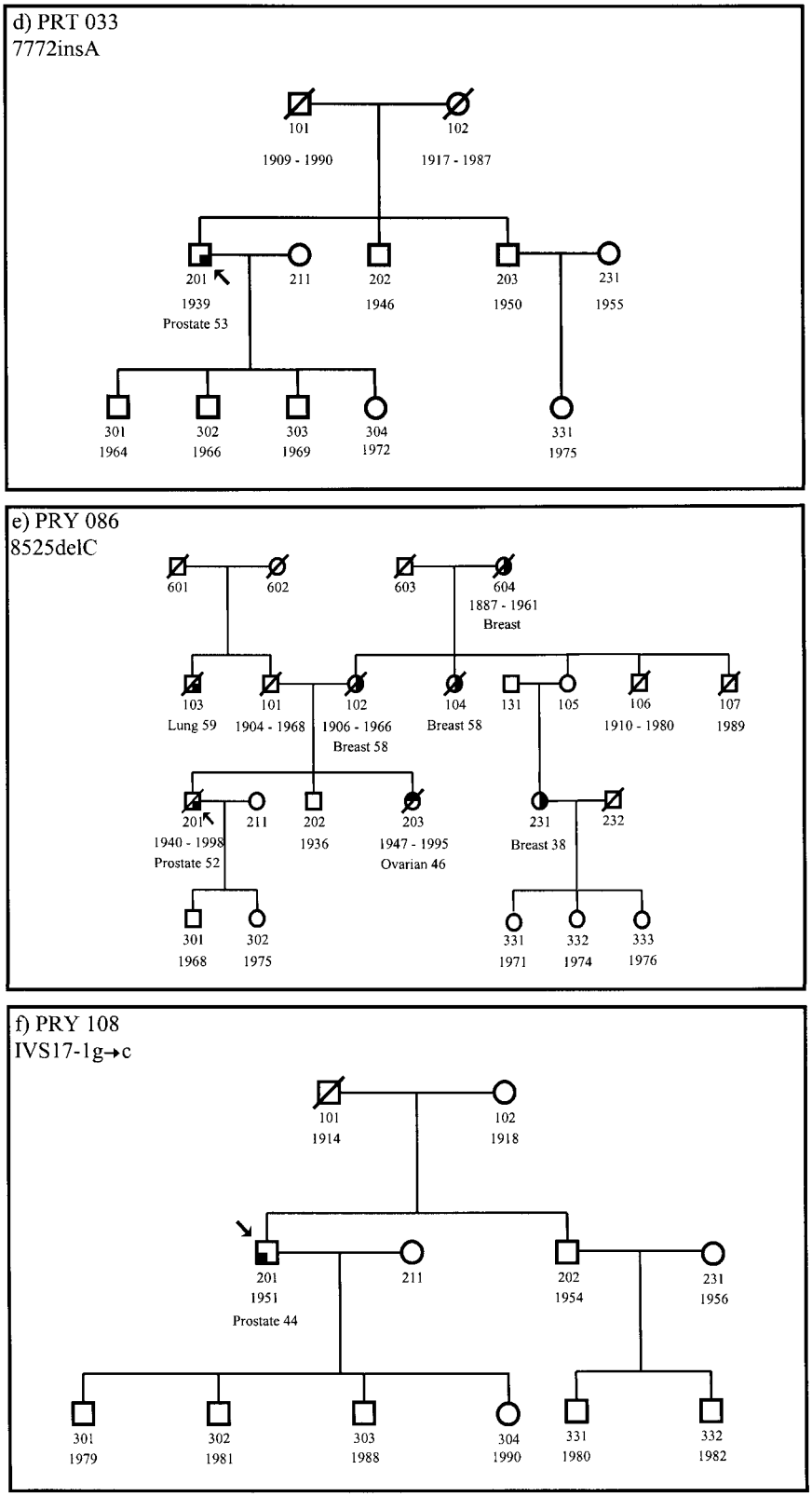


Figure 3 Pedigrees of patients with PRCA who have deleterious mutations. The index patient is indicated with an arrow. Sites of cancer and ages at diagnosis and years of birth (if available) are shown. All trees have had details of other unaffected individuals altered to preserve anonymity. These changes do not alter the inference of the study.

0.48). Mutations in this region, which is coincident with the BRC repeat domain (Bignell et al. 1997) responsible for RAD51 binding, are also associated with a reduced risk of breast cancer but a higher risk of ovarian cancer. In this regard, it is interesting to note that all six mutations found in our study lie outside the OCCR.

The positional effect noted above would also be consistent with the apparently weaker association found in studies of the Ashkenazi Jewish founder mutation 6174delT, which lies within the OCCR. Another factor may be the high prevalence of screen-detected disease in the United States. The BCLC (1999) noted that the relative risk was higher in families from Europe than those from North America, suggesting that *BRCA2* predisposes predominantly to symptomatic rather than screen-detected disease.

Of the six patients with deleterious *BRCA2* mutations, one (PRY 042) had a family history of PRCA. The overall contribution of *BRCA2* mutations to the familial aggregation of PRCA is small, however. Forty-eight patients in the study (18%) had a first-degree relative with PRCA (table 4), of which at most one quarter would occur at population incidence rates (the familial risk of PRCA in first-degree relatives is at least fourfold at ages younger than 60 years; reviewed by Eeles et al. [1999]). Therefore, we estimate that *BRCA2* mutations account for ~6% (2/[75% × 48]) of the excess familial risk of the disease. From a clinical viewpoint, therefore, a family history of PRCA alone would not be a strong indication for *BRCA2* mutation screening. On the other hand, only two patients in the study had first-degree relatives with both breast cancer diagnosed before age 60 years and ovarian cancer, one of whom had an identified mutation. Therefore, in this situation, a family history of early-onset breast cancer/ovarian cancer was, as expected, a strong predictor of *BRCA2* mutation positivity. The ascertainment by early onset of PRCA was, however, the greatest predictor of *BRCA2* mutation positivity with respect to PRCA susceptibility.

In addition to the frameshift mutations, we found 24 VUS (22 distinct variants, 2 of which were observed twice; see table 2). Twenty of these (19 distinct variants) were nonsynonymous missense mutations in the coding region, and 4 were sequence variants in the 5′-UTR and intronic regions. Of the 19 nonsynonymous VUS, 6 have not been reported to the BIC database. The overall frequency of VUS (9%) is similar to the frequency of 13% found by Myriad Genetics' sequencing of 10,000 individuals (Frank et al. 2002) and suggests that the majority of these variants are not disease associated. It is possible that a subset of the variants is deleterious, but, unfortunately, the significance of an individual missense variant is very difficult to assess. Variants may be evaluated on the basis of segregation with disease in fam-

Table 4

Family History of Prostate Cancer and Other Cancers (Data Available for 263 Patients)

	No. of Patients	Frequency (%)
Patients with PRCA family history:		
In one first-degree relative	35[a]	13.3
In two first-degree relatives	6	2.3
In three or more first-degree relatives	7	2.6
Total	48	18.2
Patients with breast and/or ovarian cancer family history:		
Ovarian cancer in one first-degree relative	11	4.2
Ovarian cancer in two first-degree relatives	2	.7
Ovarian cancer in three or more first-degree relatives	1	.4
Total	14	5.3
Breast cancer in one first-degree relative aged <60 years	15[b]	5.7
Breast cancer in two first-degree relatives aged <60 years	3	1.1
Breast cancer in three or more first-degree relatives aged <60 years	1	.4
Total	19	7.2
Breast cancer diagnosed at age <60 years and ovarian cancer in first-degree relatives	2[b]	.7
Patients with prostate and breast cancer family history:		
Breast cancer diagnosed at age <60 years and PRCA in first-degree relatives	6	2.3
Patients with any other cancer family history:		
Any cancer in one first-degree relative	79[c]	30.0
Any cancer in two first-degree relatives	38	14.4
Any cancer in three or more first-degree relatives	35	13.3
Total	152	57.7

[a] Includes PRY 042 (6710delACAA), who has a brother with PRCA.
[b] Includes PRY 086 (8525delC), who has four relatives with breast cancer and one with ovarian cancer.
[c] Includes PRY 012 (2558insA), who has a first-degree relative with laryngeal cancer.

ilies, population frequency, conservation across species, and predicted functional significance, but these are rarely definitive. We note that 13 of the 22 VUS are 100% conserved between human and mouse (overall amino acid identity between the human and mouse BRCA2 is ~60%; see table 2). Three of these (I1275M, L1457F, and N1730Y) are within the BRC repeat domain. Two of the VUS found are 100% conserved between human, mouse, and pufferfish (exon 18 D2665G and A2717S). As an illustration of the difficulties in classifying these variants, the Y42C variant detected in our study severely compromises the transcriptional activating function of the BRCA2 protein, on the basis of a yeast functional assay (Milner et al. 1997), suggesting that it may be disease associated. However, the variant has been observed to occur concurrently with other deleterious mutations and not to segregate with disease in families with breast cancer (Myriad Genetics, personal communication), demonstrating that the variant cannot be a high-risk mutation. It is, of course, possible that some of these variants or polymorphisms confer a more moderate risk of PRCA, as has been demonstrated for the N372H polymorphism and breast cancer (Healey et al. 2000).

The inconclusive nature of genetic linkage studies in PRCA suggests that the genetics of the disease are highly complex, and therefore, if mutations in other candidate PRCA susceptibility genes exist at frequencies similar to *BRCA2*, high-throughput mutation screening may be required to identify them. This study has demonstrated that *BRCA2* mutations, which are individually rare in the population, are responsible for a significant fraction of early-onset PRCA cases outside of families with multiple cases of breast-ovarian cancer, and therefore, *BRCA2* is currently the only high-risk gene for which definitive evidence of susceptibility to PRCA, from multiple studies, is available.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Authors' Web site, http://www.icr.ac.uk/cancgen/cangen/f_md_brca2_primers.htm (for primer sequences and PCR conditions [*BRCA2*])

Breast Cancer Information Core, http://research.nhgri.nih.gov/bic/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *BRCA2* [MIM 600185], *HPC1* [MIM 601518], *RNASEL* [MIM 180435], and *HPC2* [MIM 605367])

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