ONLINE MUTATION REPORT

BRCA1 and BRCA2 mutations have no major role in predisposition to prostate cancer in Finland

T Ikonen, M P Matikainen, K Syrjäkoski, N Mononen, P A Koivisto, A Rökman, E H Seppälä, O-P Kallioniemi, T L J Tammela, J Schleutker

J Med Genet 2003;40:e98(<http://www.jmedgenet.com/cgi/content/full/40/8/e98>)

After lung cancer, prostate cancer is the second leading cause of death from cancer in men in developed countries.¹ Despite the substantial impact on public health of prostate cancer, the cause of the disease has remained poorly understood, with ethnicity, diet, and family history considered as the major risk factors.^{2,3} A fraction of patients with prostate cancer belong to families with hereditary prostate cancer (HPC). Recently, several loci have been implicated in predisposing to prostate cancer by genetic linkage studies in cancer families, including *HPC1* at 1q24-q25, *HPC2* at 17p11, *PCAP* at 1q42.2-q43, *HPCX* at Xq27-q28, *CAPB* at 1p36, and *HPC20* at 20q13.⁴ Only two genes have been identified from these chromosomal regions, *ELAC2* (MIM 605367) from the *HPC2*, locus⁵ and *RNASEL* (MIM 180435) from the *HPC1*, locus⁶ but none has so far been definitively confirmed. In Finland, neither of these genes alone explains disease segregation in Finnish families with HPC but they seem to have some modifying role in predisposition to cancer.^{7,8}

Breast cancer predisposing genes *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) are involved in repair of DNA damage and transcriptional regulation.⁹ Clustering of breast, ovarian, and prostate cancer has been reported,¹⁰⁻¹⁴ suggesting a role for *BRCA1* and *BRCA2* in predisposition to prostate cancer. Several epidemiological studies have discovered an increased risk of prostate cancer among *BRCA1* and *BRCA2* families.¹⁵⁻¹⁹ Consistent with this view, loss of heterozygosity at the *BRCA1* and *BRCA2* loci is commonly encountered in prostate cancer.²⁰⁻²² Edwards *et al*²³ implicated *BRCA2* as a high risk gene for early onset prostate cancer. In a recent study in *BRCA1* and

BRCA2 mutation positive and mutation negative Finnish breast cancer families, the only increased standardised incidence ratio (SIR), besides breast and ovarian cancer, was that for prostate cancer in *BRCA2* families (SIR 4.9, 1.8-11).²⁴

To assess the contribution of *BRCA2* germline mutations to prostate cancer susceptibility in Finland, we screened seven Finnish *BRCA2* founder mutations from 444 unselected, consecutive patients with prostate cancer and from 104 patients with HPC. We also studied the role of *BRCA1* in causation of prostate cancer by screening five unique and six founder *BRCA1* mutations from 46 Finnish patients with HPC. These *BRCA1/2* founder mutations account for the vast majority of all Finnish *BRCA1/2* mutations.^{25,26} All of the *BRCA2* founder mutations indicated in the study by Eerola *et al*,²⁴ showing increased risk of prostate cancer in *BRCA2* families, were included in our analysis.

BRCA2 mutations were analysed from 444 unselected patients with prostate cancer. All of these patients, diagnosed between 1996 and 1999, came from the Tampere University Hospital, which serves as the local referral area for treatment of prostate cancer. The average age of diagnosis in these patients was 68.1 years (range 48-94 years). Also, we screened *BRCA2* mutations from 104 confirmed families with HPC. Only one affected member from each family was analysed. Collection of Finnish families with HPC has been described elsewhere.²⁷ The mean number of affected members in these families was 2.7 (range 2-6), and the mean age at diagnosis was 67.1 years (range 50-92). Confirmed breast cancer, ovarian cancer, or both cancer types were present in 20, three, and two families, respectively. Six women being first degree relatives to the HPC index, and diagnosed with breast cancer or ovarian cancer, were also analysed for *BRCA2* mutations. From 46 Finnish families with HPC, 11 *BRCA1* mutations were also screened. This subset of families represented mainly the most extreme HPC families and had a reduced number of smaller families compared to the whole collection of 104 Finnish HPC families. The mean number of affected members in these families was 3.1 (range 2-6), and the mean age at diagnosis was 66.1 years (range 50-82). The patients' diagnoses and family histories were obtained from questionnaires and subsequently confirmed from the medical records, from the Finnish Cancer Registry, and from parish records. Written informed consent was obtained from all living patients and, in hereditary cases, also from family members. The research protocols were approved by the ethics committee of the Tampere University Hospital (93175, 95062, and 99228). The study of HPC was also approved by the Ministry of Social

Key points

- Familial clustering of breast cancer, ovarian cancer, and prostate cancer has often been reported, suggesting that the breast cancer predisposition genes, *BRCA1* and *BRCA2*, may play a part in causing prostate cancer. Recent studies have implicated *BRCA2* as a high risk gene for early onset prostate cancer. Epidemiological analysis in Finnish breast cancer families discovered an excess risk of prostate cancer in *BRCA2* families.
- To assess the contribution of *BRCA2* germline mutations to causation of prostate cancer in Finland, seven Finnish *BRCA2* founder mutations from 444 unselected patients with prostate cancer and 104 hereditary cases of prostate cancer were screened.
- We also studied the role of *BRCA1* in causation of prostate cancer by screening 11 *BRCA1* mutations from 46 Finnish patients with HPC. We did not find any *BRCA1* or *BRCA2* mutations, indicating that the recurrent *BRCA1* and *BRCA2* mutations have no role in predisposition to prostate cancer in Finland.

Abbreviations: ASO, allele specific oligonucleotide hybridisation; *BRCA1* and *BRCA2*, breast cancer predisposing genes; HPC, hereditary prostate cancer; PCR, polymerase chain reaction; SIR, standardised incidence ratio

Table 1 *BRCA1* and *BRCA2* mutations screened from patients with unselected PRCA or HPC

Gene and mutation	Type of mutation	Status in Finland
<i>BRCA1</i>		
Ex 11, 1924 delA	Frameshift	Unique
Ex 11, 2803 delAA	Frameshift	Founder
Ex 11, 3604 delA	Frameshift	Founder
Ex 11, 3744 delT	Frameshift	Founder
Ex 11, 3904 C>A	Nonsense	Unique
Ex 11, 4153 delA	Frameshift	Unique
Int 11, 4216 (-2)A>G	Splice site	Founder
Ex 13, 4446 C>T	Nonsense	Founder
Ex 17, 5145 del 11 bp	Frameshift	Unique
Ex 20, 5370 C>T	Nonsense	Founder
Ex 20, 5385 insC	Frameshift	Unique
<i>BRCA2</i>		
Ex 9, 999 del5	Frameshift	Founder
Ex 11, 4081 insA	Frameshift	Founder
Ex 11, 5797 G>T	Nonsense	Founder
Ex 11, 6503 delTT	Frameshift	Founder
Ex 15, 7708 C>T	Nonsense	Founder
Ex 18, 8555 T>G	Nonsense	Founder
Int 23, 9346 (-2)A>G	Splice site	Founder

Affairs and Health (59/08/95) and the National Human Genome Research Institute Institutional Review Board (HG-0158). DNA was extracted from whole blood using the Puregene kit (Gentra Systems, Minneapolis, MN) and the manufacturer's protocol.

The frequencies of the *BRCA1* and *BRCA2* mutations (table 1) were determined by minisequencing²⁸ or by allele specific oligonucleotide hybridisation (ASO) analysis. All primers are available on request from the authors. Polymerase chain reaction (PCR) was performed with 100 ng DNA, 0.2 µmol/l of each primer, 0.2 mmol/l of each dNTP, 1.5–2.0 mmol/l MgCl₂, and 1.25–2.5 U AmpliTaqGold (PE Biosystems, Foster City, CA, USA), in a final volume of 50 µl. The ASO hybridisation analysis was performed using 5 µl of DNA product from each PCR reaction, which was mixed with 55 µl of denaturation solution (0.4 mol/l NaOH, 25 mmol/l EDTA pH 8.0) in a 98 well plate format. Each sample (50 µl) was loaded into a 48 well slot blot vacuum apparatus containing a prewetted Gene Screen Plus (DuPont NEN) nylon filter (0.4 mol/l Tris HCl pH 7.5 for 10 minutes) and vacuum was applied. Wells were washed with 100 µl of 0.4 mol/l Tris-HCl pH 7.5 under vacuum. DNA was fixed onto the filter by exposure to UV light for two minutes. Oligonucleotides specific to each mutation and its wild type sequence were end labelled with ³²P at 37°C for three hours. Labelling reaction (6.25 µl) contained 40 ng oligonucleotide, 9 units of terminal deoxynucleotidyl transferase (Amersham), 100 mmol/l sodium cocidylate pH 7.2, 0.2 mmol/l 2-mercaptoethanol, and 2 mmol/l CoCl₂. Labelled oligonucleotide was purified using NucTrap Probe purification columns (Stratagene) according to the manufacturer's instructions. Filters were incubated with 7 ml hybridisation solution containing 3 mol/l tetramethylammonium chloride, 50 mmol/l Tris HCl pH 8.0, 2 mmol/l EDTA pH 8.0, 5 × Denhardt's solution, 0.1% SDS, and 100 µg/ml herring sperm DNA at 54°C for one hour. The ASO probe (150 000 cpm/ml) was then added and the mixture incubated at 54°C for three hours. Filters were washed for 30 minutes in 2 × SSPE, 0.1% SDS, and then with 3 mol/l tetramethylammonium chloride, 50 mmol/l Tris HCl pH 8.0, 2 mmol/l EDTA pH 8.0, and 0.1% SDS for 10–30 minutes at temperatures rising from 20°C to 60°C. Filters were air dried for five minutes and exposed to x ray film overnight.

We screened 444 unselected patients with prostate cancer and 104 patients with confirmed HPC for seven Finnish *BRCA2* founder mutations and 46 patients with HPC for 11 *BRCA1* mutations of which six are recurrent in Finland.^{25–26} In the

analysis, no mutations were found, suggesting a limited role for *BRCA1* and *BRCA2* in causing prostate cancer in Finland. However, this conclusion should be treated cautiously as the samples were not screened for new mutations. The negative result was surprising as all the *BRCA2* founder mutations indicated by Eerola *et al*²⁴ in the study showing increased risk of prostate cancer in Finnish *BRCA2* families were included in our mutation screening. Our result indicates that if *BRCA2* has a role in predisposition to prostate cancer in Finland it is contributed to by other variations than known *BRCA2* Finnish founder mutations. Interestingly, Matikainen *et al*²⁹ showed no association between prostate and breast cancer in a population based cancer registry study in Finland. Besides prostate cancer, gastric cancer was the only cancer type with a significantly increased risk among the relatives of patients with prostate cancer.

The effect of individual risk genes for complex diseases could be identified more readily in isolated and genetically homogeneous populations, such as the Finnish,^{30–31} the Ashkenazi Jewish, and the Icelandic populations than in more heterogeneous ones. Among the Ashkenazi Jewish population, similar results for a minor role of *BRCA1/BRCA2* in causation of prostate cancer have been reported.^{32–34} In Iceland, however, contradictory results have been presented about the role of *BRCA2* founder mutation 999del5 in predisposition to prostate cancer.^{35–37} Our negative results stand in line with the recent studies in more mixed populations, such as the United Kingdom and the Mayo Clinic in Minnesota, which suggested a minor role for *BRCA1* and *BRCA2* in HPC,^{38–39} indicating that the result is not merely a population based phenomenon. Although some studies suggested that *BRCA2* may have a role in predisposing to early onset prostate cancer, with or without a family history of cancer,^{23–38–40} we found no evidence for that in our study. A significant number of patients with early onset prostate cancer (age at diagnosis <60 years; 18.3% among patients with HPC, and 16.2% among unselected cases of prostate cancer) were analysed but no *BRCA2* mutations were found. Further support for our negative result comes from the recently performed genome wide linkage study using Finnish families with HPC, which did not show any linkage to 17q or 13q (J Schleutker, unpublished data), the chromosomal sites of *BRCA1* and *BRCA2*, respectively.^{41–43}

In conclusion, our results indicate that the recurrent *BRCA1* and *BRCA2* mutations have a limited, if any, role in predisposition to prostate cancer in Finland.

ACKNOWLEDGEMENTS

We thank Minna Sjoblom, Kati Rouhento, Kaisa Vaherto, and Eeva Rauhalala for excellent technical assistance. Dr Heli Nevanlinna is thanked for providing positive control for *BRCA2* 4081insA mutation screening. The patients with prostate cancer and their families are thanked for their participation and cooperation. The present study was supported by grants from the Medical Research Fund of the Tampere University Hospital, and the Academy of Finland. Ascertainment of families with HPC in Finland was supported by grant NO1-HG-55389 from the National Human Genome Research Institute, National Institutes of Health.

Authors' affiliations

T Ikonen, K Syrjäkoski, N Mononen, P A Koivisto, A Rökman, E H Seppälä, J Schleutker, Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital, Finland
M P Matikainen, T L J Tammela, Department of Urology, Tampere University Hospital and Medical School, Tampere, Finland
O-P Kallioniemi, Medical Biotechnology Group, Technical Research Centre of Finland and University of Turku, Turku, Finland

Correspondence to: Dr T Ikonen, Laboratory of Cancer Genetics, Institute of Medical Technology, FIN-33014 University of Tampere, Finland; tarja.ikonen@uta.fi

REFERENCES

- 1 Penttala SN, Lee J, Hsieh K, Waltzer WC, Trocchia A, Musacchia L, Rebecchi MJ, Khan SA. Prostate cancer: a comprehensive review. *Med Oncol* 2000;17:85–105.

- 2 **Pienta KJ**, Esper PS. Risk factors for prostate cancer. *Ann Intern Med* 1993;**118**:793–803.
- 3 **Rowley KH**, Mason MD. The aetiology and pathogenesis of prostate cancer. *Clin Oncol* 1997;**9**:213–18.
- 4 **Ostrander EA**, Stanford JL. Genetics of prostate cancer: too many loci, too few genes. *Am J Hum Genet* 2000;**67**:1367–75.
- 5 **Tavtigian SV**, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp, NJ, Carillo AR, Chen, Y, Dayananth P, Desrochers M, Dumont M, Farnham JM, Frank D, Frye C, Ghaffari S, Gupte JS, Hu R, Iliev D, Janecki T, Kort EN, Laity KE, Leavitt A, Leblanc G, McArthur-Morrison J, Pederson A, Penn B, Peterson KT, Reid JE, Richards S, Schroeder M, Smith R, Snyder SC, Swedlund B, Swensen J, Thomas A, Tranchant M, Woodland AM, Labrie F, Skolnick MH, Neuhausen S, Rommens J, Cannon-Albright LA. A candidate prostate cancer susceptibility gene at chromosome 17p. *Nat Genet* 2001;**27**:172–80.
- 6 **Carpenter J**, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallioniemi OP, Burk R, Meyers D, Gronberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W, Trent J. Germline mutations in the ribonuclease L gene in families showing linkage with HPC. *Nat Genet* 2002;**30**:181–4.
- 7 **Rokman A**, Ikonen T, Mononen N, Autio V, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J. ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer. *Cancer Res* 2001;**61**:6038–41.
- 8 **Rokman A**, Ikonen T, Seppala EH, Nupponen N, Autio V, Mononen N, Bailey-Wilson J, Trent J, Carpenter J, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J. Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. *Am J Hum Genet* 2002;**70**:1299–304.
- 9 **Welsh PL**, King MC. BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Hum Mol Genet* 2001;**10**:705–13.
- 10 **Tulinius H**, Egilsson V, Olafsdottir GH, Sigvaldason H. Risk of prostate, ovarian and endometrial cancer among relatives of women with breast cancer. *BMJ* 1992;**305**:855–7.
- 11 **Arason A**, Barkardottir RB, Egilsson V. Linkage analysis of chromosome 17q markers and breast-ovarian cancer in Icelandic families, and possible relationship to prostatic cancer. *Am J Hum Genet* 1993;**52**:711–17.
- 12 **Anderson DE**, Badzioch MD. Familial effects of prostate and other cancers on lifetime breast cancer risk. *Breast Cancer Res Treat* 1993;**28**:107–13.
- 13 **Sellers TA**, Potter JD, Rich SS, Drinkard CR, Bostick RM, Kushi LH, Zheng W, Folsom AR. Familial clustering of breast and prostate cancers and risk for postmenopausal breast cancer. *J Natl Cancer Inst* 1994;**86**:1860–5.
- 14 **Easton DF**, Steele L, Fields P, Ormiston W, Averill D, Daly PA, McManus R, Neuhausen SL, Ford D, Wooster R, Cannon-Albright LA, Stratton MR, Goldgar DE. Cancer risks in two large breast cancer families linked to BRCA2 on chromosome 13q12–13. *Am J Hum Genet* 1997;**61**:120–8.
- 15 **Ford D**, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. *Lancet* 1994;**343**:692–5.
- 16 **Struwing JP**, Hartge P, Wacholder S, Bacer SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med* 1997;**336**:1401–8.
- 17 **The Breast Cancer Linkage Consortium**. Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* 1999;**91**:1310–16.
- 18 **Johansson O**, Loman N, Moller T, Kristofferson U, Borg A, Olsson H. Incidence of malignant tumours in relatives of BRCA1 and BRCA2 germline mutation carriers. *Eur J Cancer* 1999;**35**:1248–57.
- 19 **Warner E**, Foulkes W, Goodwin P, Meschino W, Blondal J, Paterson C, Ozcelik H, Goss P, Allingham-Hawkins D, Hamel N, Di Prospero L, Contiga V, Serruya C, Klein M, Moslehi R, Honeyford J, Liede A, Glendon G, Brunet JS, Narod S. Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. *J Natl Cancer Inst* 1999;**91**:1241–7.
- 20 **Gao X**, Zacharek A, Salkowski A, Grignon DJ, Sakr W, Porter AT, Honn KV. Loss of heterozygosity of the BRCA1 and other loci on chromosome 17q in human prostate cancer. *Cancer Res* 1995;**55**:1002–5.
- 21 **Melamed J**, Einhorn JM, Littmann MM. Allelic loss on chromosome 13q in human prostate carcinoma. *Clin Cancer Res* 1997;**3**:1867–72.
- 22 **Edwards SM**, Dunsmuir WD, Gillett CE, Lakhani SR, Corbishley C, Young M, Kirby RS, Dearnaley DP, Dowe A, Ardern-Jones A, Kelly J, Spurr N, Barnes DM, Eeles RA. Immunohistochemical expression of BRCA2 protein and allelic loss at the BRCA2 locus in prostate cancer. *Int J Cancer* 1998;**78**:1–7.
- 23 **Edwards SM**, Kote-Jarai Z, Meitz J, Hamoudi R, Hope Q, Osin P, Jackson R, Southgate C, Singh R, Falconer A, Dearnaley DP, Ardern-Jones A, Murkin A, Dowe A, Kelly J, Williams S, Oram R, Stevens M, Teare DM, Ponder BAJ, Gayther SA, The Cancer Research UK/British Prostate Group, UK Familial Prostate Cancer Study Collaborators, British Association of Urological Surgeons Section of Oncology, Easton DF, Eeles RA. Two per cent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene. *Am J Hum Genet* 2003;**72**:1–12.
- 24 **Eerola H**, Pukkala E, Pyyhonen S, Blomqvist C, Sankila R, Nevanlinna H. Risk of cancer in BRCA1 and BRCA2 mutation-positive and -negative breast cancer families (Finland). *Cancer Causes Control* 2001;**12**:739–46.
- 25 **Sarantausta L**, Huusko P, Eerola H, Launonen V, Vehmanen P, Rapakko K, Gillanders E, Syrjäkoski K, Kainu T, Vahteristo P, Krahe R, Paakkonen K, Hartikainen J, Blomqvist C, Loppinen T, Holli K, Ryyanen M, Butzow R, Borg A, Wasteson Arver B, Holmberg E, Mannermaa A, Kere J, Kallioniemi O-P, Winqvist R, Nevanlinna H. Multiple founder effects and geographical clustering of BRCA1 and BRCA2 families in Finland. *Eur J Hum Genet* 2000;**8**:757–63.
- 26 **Syrjäkoski K**, Vahteristo P, Eerola H, Tamminen A, Kivunniemi K, Sarantausta L, Holli K, Blomqvist C, Kallioniemi O-P, Kainu T, Nevanlinna H. Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. *J Natl Cancer Inst* 2000;**92**:1529–31.
- 27 **Schleutker J**, Matikainen M, Smith J, Koivisto P, Baffoe-Bonnie A, Kainu T, Gillanders E, Sankila R, Pukkala E, Carpten J, Stephan D, Tammela T, Brownstein M, Bailey-Wilson J, Trent J, Kallioniemi O-P. A genetic epidemiological study of hereditary prostate cancer (HPC) in Finland: frequent HPCX linkage in families with late-onset disease. *Clin Cancer Res* 2000;**6**:4810–15.
- 28 **Syvanen AC**. Solid-phase minisequencing as a tool to detect DNA polymorphism. *Methods Mol Biol* 1998;**98**:291–8.
- 29 **Matikainen MP**, Pukkala E, Schleutker J, Tammela TL, Koivisto P, Sankila R, Kallioniemi O-P. Relatives of prostate cancer patients have an increased risk of prostate and stomach cancers: a population-based, cancer registry study in Finland. *Cancer Causes Control* 2001;**12**:223–30.
- 30 **Peltonen L**. Molecular background of the Finnish disease heritage. *Ann Med* 1997;**29**:553–6.
- 31 **de la Chapelle A**, Wright FA. Linkage disequilibrium mapping in isolated populations: the example of Finland revisited. *Proc Natl Acad Sci USA* 1998;**95**:12416–23.
- 32 **Lehrer S**, Fodor F, Stock R, Stone NN, Eng C, Song HK, McGovern M. Absence of 185delAG mutation of the BRCA1 gene and 6174delT mutation of the BRCA2 gene in Ashkenazi Jewish men with prostate cancer. *Br J Cancer* 1998;**78**:771–3.
- 33 **Wilkins EP**, Freije D, Xu J, Nusskern DR, Suzuki H, Isaacs DS, Wiley K, Bujnovszky P, Meyers DA, Walsh PC, Isaacs WB. No evidence for a role of BRCA1 and BRCA2 mutations in Ashkenazi Jewish families with hereditary prostate cancer. *Prostate* 1999;**39**:280–4.
- 34 **Nastiuk KL**, Mansukhani M, Terry MB, Kularatne P, Rubin MA, Melamed J, Gammon MD, Littmann MM, Krolewski JJ. Common mutations in BRCA1 and BRCA2 do not contribute to early prostate cancer in Jewish men. *Prostate* 1999;**40**:172–7.
- 35 **Johannesdottir G**, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA, Eiriksdottir G, Johannsson OT, Borg A, Ingvarsson S, Easton DF, Egilsson V, Barkardottir RB. High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res* 1996;**56**:3663–5.
- 36 **Thorlacius S**, Sigurdsson S, Bjarnadottir H, Olafsdottir G, Jonasson JG, Tryggvadottir L, Tulinius H, Eyfjord JE. Study of single BRCA2 mutation with high carrier frequency in a small population. *Am J Hum Genet* 1997;**60**:1079–84.
- 37 **Sigurdsson S**, Thorlacius S, Tomasson J, Tryggvadottir L, Benediktsson K, Eyfjord JE, Jonsson E. BRCA2 mutation in Icelandic prostate cancer patients. *J Mol Med* 1997;**75**:758–61.
- 38 **Gayther SA**, de Foy KAF, Harrington P, Pharoah P, Dunsmuir WD, Edwards SM, Gillett C, Ardern-Jones A, Dearnaley DP, Easton DF, Ford D, Shearer RJ, Kirby RS, Dowe AL, Kelly J, Stratton MR, The Cancer Research Campaign/British Prostate Group, United Kingdom Familial Prostate Cancer Study Collaborators, Ponder BAJ, Barnes D, Eeles RA. The frequency of germline mutations in the breast cancer predisposition genes BRCA1 and BRCA2 in familial prostate cancer. *Cancer Res* 2000;**60**:4513–18.
- 39 **Sinclair CS**, Berry R, Schaid D, Thibodeau SN, Couch FJ. BRCA1 and BRCA2 have limited a role in familial prostate cancer. *Cancer Res* 2000;**60**:1371–5.
- 40 **Gronberg H**, Ahman AK, Emanuelsson M, Bergh A, Damber JE, Borg A. BRCA2 mutation in a family with hereditary prostate cancer. *Genes Chrom Cancer* 2001;**30**:299–301.
- 41 **Miki Y**, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rostek P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;**266**:66–71.
- 42 **Wooster R**, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G, Barfoot R, Hamoudi R, Patel S, Rice C, Biggs P, Hashim Y, Smith A, Connor F, Arason A, Gudmundsson J, Ficencic D, Kelsell D, Ford D, Tonin P, Bishop DT, Spurr NK, Ponder BAJ, Eeles R, Peto J, Devilee P, Cornelisse C, Lynch H, Narod S, Lenoir G, Egilsson V, Barkardottir RB, Easton DF, Bentley DR, Futreal PA, Ashworth A, Stratton MR. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995;**378**:789–92.
- 43 **Tavtigian SV**, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, Thorlacius S, Offit K, Stoppa-Lyonnet D, Belanger C, Bell R, Berry S, Bogden R, Chen Q, Davis T, Dumont M, Frye C, Hattier T, Jammulapati S, Janecki T, Jiang P, Kehrer R, Leblanc J-F, Mitchell JY, McArthur-Morrison J, Nguyen K, Peng Y, Samson C, Schroeder M, Snyder SC, Steele L, Stringfellow M, Stroup C, Swedlund B, Swensen J, Teng D, Thomas A, Tran T, Tranchant M, Weaver-Feldhaus J, Wong AKC, Shizuya H, Eyfjord JE, Cannon-Albright L, Labrie F, Skolnick MH, Weber B, Kamb A, Goldgar DE. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 1996;**12**:333–7.