After lung cancer, prostate cancer is the second leading cause of death from cancer in men in developed countries. 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. 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. Only two genes have been identified from these chromosomal regions, ELAC2 (MIM 605367) from the HPC2, locus and RNASEL (MIM 180435) from the HPC1, locus 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.

Breast cancer predisposing genes BRCA1 (MIM 113705) and BRCA2 (MIM 600185) are involved in repair of DNA damage and transcriptional regulation. Clustering of breast, ovarian, and prostate cancer has been reported, 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. Consistent with this view, loss of heterozygosity at the BRCA1 and BRCA2 loci is commonly encountered in prostate cancer. Edwards et al 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).

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. All of the BRCA2 founder mutations indicated in the study by Eerola et al., 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. 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

<table>
<thead>
<tr>
<th>Gene and mutation</th>
<th>Type of mutation</th>
<th>Status in Finland</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex 11, 1924 delA</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>Ex 11, 2803 delAA</td>
<td>Frameshift</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 11, 3604 delA</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>Ex 11, 3744 delF</td>
<td>Frameshift</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 11, 3904 C&gt;A</td>
<td>Nonsense</td>
<td>Unique</td>
</tr>
<tr>
<td>Ex 11, 4153 delA</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>Int 11, 4216 (2)A&gt;G</td>
<td>Splice site</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 13, 4446 C&gt;T</td>
<td>Nonsense</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 17, 5145 del 11 bp</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>Ex 20, 5370 C&gt;T</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>Ex 20, 5385 insC</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex 9, 999 delS</td>
<td>Frameshift</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 11, 4081 insA</td>
<td>Frameshift</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 11, 5797 G&gt;T</td>
<td>Nonsense</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 11, 6503 delT</td>
<td>Frameshift</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 15, 7708 C&gt;T</td>
<td>Nonsense</td>
<td>Founder</td>
</tr>
<tr>
<td>Ex 18, 8555 insG</td>
<td>Frameshift</td>
<td>Unique</td>
</tr>
<tr>
<td>Int 23, 9346 (2)A&gt;G</td>
<td>Splice site</td>
<td>Founder</td>
</tr>
</tbody>
</table>

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 minisequencing15 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 µmol/l of each dNTP, 1.5–2.0 µmol/l MgCl₂, and 1.25–2.5 U AmpliTagGold (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. A mixture (50 µl) was loaded into a 48 well wall slot 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 nylon filter by exposure to UV light for two minutes. Oligonucleotides specific to each mutation and its wild type fixed onto the filter by exposure to UV light for two minutes. (DuPont NEN) nylon filter (0.4 mol/l Tris HCl pH 7.5 for 10 minutes) and the mixture incubated at 54°C for three hours. 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, and 100 µg/ml herring sperm DNA at 37°C for two hours. Labelling reaction (6.25 µl of 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 nylon filter by exposure to UV light for two minutes. Oligonucleotides specific to each mutation and its wild type sequence were end labelled with 'P 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 cacodylate 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 ranging 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 140 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.16 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 al12 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 al13 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,11 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.11,12 In Iceland, however, contradictory results have been presented about the role of BRCA2 founder mutation 999del5 in predisposition to prostate cancer.11,12 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.11,12 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,11,12,14 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.11,12

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 Raunala for excellent technical assistance. Dr Heli Nevanlinna is thanked for providing positive control for BRCA2 401insA 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 Biotechnology Group, Technical Research Centre of Finland and University of Turku, Turku, Finland

Departments of Oncology and Laboratory Medicine of Medical Technology, FIN-33014 University of Tampere, Finland; on June 7, 2022 by guest. Protected by copyright.http://jmg.bmj.com/ J Med Genet: first published as 10.1136/jmg.40.8.e98 on 14 August 2003. Downloaded from

REFERENCES


2 BRCA1

3 BRCA2


Online mutation report