BRCA2 germline mutations among early onset breast cancer patients unselected for family history of the disease

Editor—Germline mutations in the BRCA1 and BRCA2 genes are associated with an increased risk of breast and ovarian cancer.1–3 Different patient populations, predominantly with at least some familial aggregation of breast/ovarian cancer, have been screened to estimate the frequency of mutations and identify women for whom mutation analysis should be considered. The prevalence of BRCA1 and BRCA2 gene mutations has been studied in only a few population based series of patients.4–8

We tested for BRCA2 germline mutations in 40 patients diagnosed with breast cancer before the age of 40, unselected for family history of the disease. These patients were drawn from breast cancer patients recruited between 1992 and 1995 in a population based genetic epidemiological case-control study conducted in a region around Heidelberg in Germany.9 The mean age of onset of the disease in the tested population was 33.8 years (range 25–39 years). Written informed consent was obtained from all patients. The 26 coding exons and flanking intronic regions were analysed from genomic DNA using published10 11 or newly designed primers (available on request). Polymerase chain reactions contained 50 ng DNA, 200 mmol/l of each dinucleotide, 1.5-3.0 mmol/l MgCl₂, 200 nmol/l of each primer, and 1 unit of AmpliTaq (PE Applied Biosystems, Weiterstadt, Germany) in a total volume of 25 µl. Conditions were one minute at 94°C, one minute at 55°C-62°C, and one or two minutes at 72°C for 35 cycles with five minutes at 94°C before and seven minutes at 72°C after cycling. Amplified fragments were excised from agarose gels, eluted in sterile water, and sequenced directly, applying the Thermo Sequenase™ Fluorescent Cycle Sequencing kit (Amersham Pharmacia Biotech, Freiburg, Germany) according to the manufacturer’s protocol. Sequencing primers were those used for exon amplification or additional internal primers (44 sequencing reactions/patient), and were Cy5™ labelled, allowing sequence analysis on ALF express devices (Amersham Pharmacia Biotech). Electrophoresis was performed according to standard protocols.

Five mutations predicted to lead to a truncated protein and three rare missense variants were identified (table 1). A pathogenic role of the rare missense variants remains to be clarified. The conservation of the isoleucine residue at codon 729 extends to monkey and dog, but not to hamster and mouse homologues.12 The threonine residue at codon 2515 is replaced in mouse and rat by a similar serine,13 and is changed in one of our patients to a non-conserved isoleucine, whereas the valine residue at codon 2728 is conserved among the three species and is changed to a similar isoleucine in the patient. Neither of the latter variants has so far been found in control populations but only in patients.14 15 To our knowledge, the amino acid replacement at codon 729 and the 4 bp deletion at nucleotide 4875 (fig 1)

<table>
<thead>
<tr>
<th>Case</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Type*</th>
<th>Age</th>
<th>Affected family members†</th>
<th>Parental side‡</th>
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<tr>
<td>HdB5</td>
<td>10</td>
<td>2041insA</td>
<td>615ter</td>
<td>F</td>
<td>31</td>
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<td>6710delACAA</td>
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<tr>
<td>HdB90</td>
<td>25</td>
<td>C9610T</td>
<td>Arg128Stop</td>
<td>N</td>
<td>29</td>
<td>Mother at 52, aunt (P) at 48</td>
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<td>A2415G</td>
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<td>Thr2515Ile</td>
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<td>G8410A</td>
<td>Val2728Ile</td>
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<td>34</td>
<td>None</td>
<td>M</td>
</tr>
</tbody>
</table>

*F: frameshift; N: nonsense; M, missense.
†P, paternal; M, maternal.
‡Proven by available parental blood samples, otherwise ND, not defined.

Figure 1 Novel BRCA2 sequence alterations found in cases HdB71 and HdB88.
have not yet been reported. In addition, we detected five amino acid polymorphisms (4 × N289H, 11 × H372N, 4 × N991D, 1 × T1915M, 3 × I3412V) and six silent and six intronic sequence variants.

Only one of the five mutation carriers and none of the three patients harbouring a rare missense variant reported additional family members with breast/ovarian cancer. We were able to attribute this partly to paternal transmission of the mutation. Parental blood samples were available in two cases with truncating mutations (HdB5, HdB28) and the mutation was found to be transmitted from the father. A low penetrance of mutations in some families, as has been reported in population based studies and for an Icelandic BRCA2 founder mutation, may also explain an absence of family history of the disease.

Peto et al. detected BRCA2 mutations using conformation sensitive gel electrophoresis in 2.3% of 617 women diagnosed before 46 years of age from two population series of breast cancer patients in Britain. In a population based sample of 388 Australian women diagnosed with breast cancer before the age of 40, BRCA2 mutations, detected by the protein truncation test, were estimated to account for about 3% of cases. In North American based studies, BRCA2 mutations were found in 3.4% of a population based sample of 203 patients diagnosed before the age of 35 years and in 2.7% of a hospital based series of 73 patients diagnosed before the age of 32 years. The frequency in our study population was 12.5% (exact 95% CI 9.5-16.2%) when only German patients were considered. In 90 non-Ashkenazi North American patients aged under 40 with a family history of breast/ovarian cancer, 9% was found in 90 non-Ashkenazi North American patients aged under 40 with a family history of breast/ovarian cancer. Assuming a pathogenic role of one or several of the rare missense variants and, possibly, regulatory mutations or rearrangements of larger genomic regions, which were not screened for in our study, the proportion of breast cancer by the age of 40 which can be attributed to a BRCA2 mutation in Germany may be higher.

Our data indicate that the probability of harbouring a BRCA2 mutation among early onset breast cancer patients in the population is relatively independent of a reported positive family history for the disease, as also observed by other colleagues. Owing to the sex differential in breast cancer risk, selection by family history generally leads to an over-representation of inherited disease transmitted along the maternal lineage and an underdetection of predisposition of paternal origin among patients with mutations. Family history does not appear to be a good predictor of genetic susceptibility for early onset breast cancer.

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