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A probability model for predicting *BRCA1* and *BRCA2* mutations in breast and breast-ovarian cancer families

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Summary Germline mutations in *BRCA1* and *BRCA2* genes predispose to hereditary breast and ovarian cancer. Our aim was to find associations between the clinical characteristics and positive mutation status in 148 breast cancer families in order to predict the probability of finding a *BRCA* mutation in a family. Several factors were associated with mutations in univariate analysis, whereas in multivariate analysis (logistic regression with backward selection) only the age of the youngest breast cancer patient and the number of ovarian cancer cases in a family were independent predictors of *BRCA* mutations. A logistic model was devised to estimate the probability for a family of harbouring a mutation in either *BRCA1* or *BRCA2*. Altogether, 63 out of 148 families (43%) and 28 out of 29 (97%) mutation carrier families obtained probabilities over 10%. The mean probability was 55% for mutation-positive families and 11% for mutation-negative families. The models by Couch et al (1997) and Shattuck-Eidens et al (1997) previously designed for *BRCA1* were also tested for their applicability to distinguish carrier families with mutations in either gene. The probability model should be a useful tool in genetic counselling and focusing the mutation analyses, and thus increasing also the cost-effectiveness of the genetic screening. © 2001 Cancer Research Campaign http://www.bjcancer.com

Keywords: breast cancer; ovarian cancer; *BRCA1*; *BRCA 2*; mutation; probability model

Mutations in the two breast-ovarian cancer susceptibility genes, *BRCA1* and *BRCA2*, account for a varying fraction of breast cancer families in different populations (Szabo and King, 1997). Both *BRCA1* and *BRCA2* mutations are scattered throughout the large coding regions of the genes (Breast Cancer Information Core). In admixed populations, most mutations appear uniquely in single families only, making the mutation screening laborious and expensive. Furthermore, there is also evidence of other predisposing genes (Ford et al, 1998; Kainu et al, 2000). It is, therefore, important to find the clinical risk factors that could best predict the presence of *BRCA1* and *BRCA2* mutations, so that the screening could be directed to potential mutation carrier families.

Several probability models for mutation detection have been developed. These are, however, based only on *BRCA1* (Berry et al, 1997; Couch et al, 1997; Shattuck-Eidens et al, 1997), focus on specific founder mutations in the Ashkenazi population (Foulkers et al, 1999; Hodgson et al, 1999; Hopper and Jenkins, 1999), or require information such as penetrance estimations not available in all populations (Berry et al, 1997; Parmigiani et al, 1998; Chang-Claude et al, 1999).

Here we have developed a model for predicting the presence of a *BRCA1* or *BRCA2* mutation in families with 3 or more relatives affected with breast or ovarian cancer. We also compared this model with those of Shattuck-Eidens et al (1997) and Couch et al (1997) originally designed for *BRCA1* only. Additionally, the frequency of *BRCA1/2* mutations was studied in 295 families with two affected family members to evaluate the feasibility of genetic screening in families with moderate family history.

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PATIENTS AND METHODS

The cohort studied consisted of 148 families with 3 or more 1st or 2nd degree relatives affected with breast or ovarian cancer. The families were identified by patient interviews, and full pedigrees were constructed with the confirmation of all genealogy data through the Finnish population registration as well as diagnostic data through hospital records and/or Finnish Cancer Registry as previously described (Vehmanen et al, 1997a,b; Eerola et al, 2000). Additionally, 295 breast cancer cases with one 1st degree relative affected with breast or ovarian cancer and identified in the patient cohorts described in Eerola et al (2000) were also studied. In the following, these are called small families. The family history of these cases was based on information reported by the index patient. All patients participating in the study signed an informed consent before the blood sample for the genetic analysis was taken. This study has been approved by the Ethical Committees of Departments of Obstetrics and Gynaecology, and Oncology, HUCH, and appropriate permissions were obtained from the Ministry of Social Affairs and Health in Finland.

The mutations identified by a complete mutation analysis of the whole coding sequences and exon/intron boundaries of the genes in 95 of these families have been previously reported (Vehmanen et al, 1997a,b). For 53 other families, all previously reported 18 Finnish *BRCA1* and *BRCA2* mutations (Vehmanen et al, 1997a,b; Huusko et al, 1998; Sarantaus et al, 2000), and one recently discovered new *BRCA1* mutation (3264 delT) were analysed by allele-specific oligonucleotide (ASO) (Friedman et al, 1995) hybridization or restriction fragment length polymorphism (RFLP). The RFLP analyses were designed such that incomplete

digestion would lead to a false positive hence minimizing the possibility of a false negative result. Sequences of the PCR primers and ASO probes, as well as the enzymes used for digestions are available upon request. Protein truncation test (PTT) (Hogervorst et al, 1995; Håkansson et al, 1997) of *BRCA1* exon 11 and *BRCA2* exons 10 and 11 was also used to search for new mutations in 36 families with an ovarian cancer case or a breast cancer patient diagnosed below 50 years. All positive mutation detection results were confirmed by direct sequencing using an ABI PRISM 310 Genetic Analyser and Dye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer's instructions (PE Applied Biosystems, Foster City, CA, USA).

For 295 small breast cancer families ASO and RFLP analyses were used to screen all known Finnish mutations, and direct sequencing was used to confirm the positive screening results. In previous studies, 11 recurrent founder mutations have been found to account for vast majority (84%) of all Finnish *BRCA1* and *BRCA2* families (Vehmanen et al, 1997a,b; Huusko et al, 1998). Therefore, screening of the known mutations was used to evaluate the feasibility of screening of the *BRCA1* and *BRCA2* genes in these families.

Statistical analysis

Associations between specific familial characteristics (presented in Table 1) and the presence of a *BRCA1* or *BRCA2* germline mutation were studied by univariate and multivariate analyses. For univariate analysis, Mann-Whitney and Fisher's exact tests (SPSS 8.0 for Windows) were used. Variables that were predictive of a mutation in a univariate analysis were used in a multivariate analysis (stepwise backward logistic regression, 99%), and based on that a logistic probability model for harbouring a deleterious mutation was devised.

The models by Couch et al (1997) and Shattuck-Eidens et al (1997), previously designed for estimating mutation probability in the *BRCA1* gene, were also tested in the 148 families and compared to the model developed here for their applicability to distinguish carrier families with mutations in either gene.

RESULTS AND DISCUSSION

Mutations identified

A total of 29 germline mutations was found in 148 families (19.6%), 16 in *BRCA1* (10.8%) and 13 in *BRCA2* (8.8%). In addition to previously known Finnish mutations, two new protein truncating mutations were identified (*BRCA1*, 1806 C → T and *BRCA2*, 5797 G → T). Both of these mutations were subsequently found also in other study cohorts (Syrjäkoski et al, 2000; Sarantaus L, personal communication) making the total number of recurrent mutations in Finland now 13. Altogether, 24 (86%) of the mutation-positive patients carried one of the recurrent mutations, and 5 patients unique mutations not found in other families so far in Finland.

Factors associated with positive mutation status

Several factors were associated with the presence of germline *BRCA1* or *BRCA2* mutations in the univariate analysis (Table 1). In the multivariate analysis, only two variables were still significant: the number of ovarian cancer cases in a family ($P < 0.00005$) and the age at diagnosis of the youngest breast cancer patient ($P = 0.0007$). The presence of breast and ovarian cancer in the same patient was not significant in multivariate analysis, probably because it is closely associated with ovarian cancer cases overall. Bilateral breast cancer, another factor that has been correlated with a positive mutation status by for example Shattuck-Eidens et al (1997) and Ligtenberg et al (1999), was not significant in univariate analysis and, therefore, not included in further analysis.

Families carrying a mutation in either *BRCA1* or *BRCA2* were also analysed separately (data not shown). The results were similar for both genes except for the number of breast cancer patients that was associated with a *BRCA2* mutation status in the univariate analysis. In the multivariate analysis the same variables were significant for both genes and, therefore, one common model could be used for distinguishing all mutation carriers. Early age of breast cancer onset as well as the presence of ovarian cancer in a family are thus highly characteristic for Finnish *BRCA2* families also. It is of interest to note that only one of the *BRCA2* mutations in this study was in the OCCR region where a higher risk of ovarian cancer, relative to breast cancer, has been suggested (Gayther et al, 1997; Ford et al, 1998).

Probability of identification of a mutation in the family

Based on the results from the multivariate analysis, a probability model for harbouring a deleterious mutation was devised, and can be written in the form of:

Table 1 Variables tested and the associations found in univariate analysis

	BRCA1 / BRCA2	non-*BRCA1/2*	P value in univariate analysis
Variables concerning the number of breast and ovarian cancer cases	Mean number of cancer cases		
Mean number of breast cancer cases in a family	3.5	3.8	0.304
Mean number of ovarian cancer cases in a family	1.4	0.2	<0.0005
Mean number of bilateral breast cancer cases in a family	0.5	0.3	0.292
Variables concerning the age at diagnosis	Age in years		
Age at diagnosis of the index case	41.3	51.4	<0.0005
Age at diagnosis of the youngest breast cancer patient	38.5	46.0	<0.0005
Age at diagnosis of the youngest ovarian cancer patient	52.0	59.7	0.056
Mean age at diagnosis of the breast cancer cases	47.6	56.4	<0.0005
Variables concerning the presence of different cancer types	Proportion		
Presence of ovarian cancer in a family	79% (23/29)	20% (24/119)	<0.0005
Presence of breast and ovarian cancer in the same individual	34% (10/29)	2.5% (3/119)	<0.0005
Presence of bilateral breast cancer in a family	31% (9/29)	24% (29/119)	0.482
Presence of prostate cancer in a family	24% (7/29)	15% (18/119)	0.272

$$p = e^L / (1 + e^L)$$

and L can be calculated from the equation $L = 2.87 + (-0.14) \times V_1 + 2.11 \times V_2$ where 2.87 is a constant and –0.14 and 2.11 are the coefficients received from the regression analysis, V_1 is the age of the youngest breast cancer patient in a family, and V_2 is the number of ovarian cancer cases in a family.

Among the 148 study families, 97% (28/29) of the mutation carrier families obtained a probability greater than an arbitrary cut off value of 10%. The mean probability was 55% for mutation-positive families and 11% for mutation-negative families. Altogether, out of 148 families 63 (43%) obtained probabilities over 10% and among these, 28 (44%) were mutation carrier families. Thus by using this model, mutation screening could be directed to a significantly smaller proportion of families.

Similar results were obtained also with the models of Shattuck-Eidens et al (1997) and Couch et al (1997) originally designed for *BRCA1* (Table 2). Thus these models distinguish also *BRCA2* mutation carrier families very efficiently. The one mutation-positive family missed in all 3 models has 3 affected breast cancer patients all diagnosed at later age. The proportion of mutations found is higher in the model developed in this study since it has been designed particularly for this study cohort, and the determination of sensitivity as well as specificity of this model requires analysis of a separate test population. The model here was also designed to estimate the carrier probability of a family with 3 or more affected cases, and therefore it could not be extrapolated to cases with a less profound family history.

Mutation frequencies in families with defined family history of cancer

All families classified by the family history of breast and ovarian cancer as well as age of breast cancer onset (below 40 years) are presented in Table 3. By analysing mutation-positive and -negative families, initially chosen by the criterion of at least 3 breast or ovarian cancer patients among 1st or 2nd degree relatives, we noted that mutation carrier families could be identified by a simple criterion of a breast cancer case diagnosed before the age of 40 or an ovarian cancer case in the family. Altogether, 80/148 (54% of all) families fulfilled this criterion, and among these, 28/29 (97%) of the mutations could be found. This simple criterion alone could thus be used as a rough estimation of a high likelihood of carrying a mutation in such families.

No mutations were found in 21 families with 4 or more cases of breast but no ovarian cancer or young breast cancer patient (diagnosis below 40 years). This is in agreement with our results from

Table 2 Comparison of the different probability models

	Shattuck-Eidens	Couch	This study
Mutation positive families identified (total)	27/29 (93%)	25/29 (86%)	28/29 (97%)
BRCA1-positive families identified	15/16 (94%)	14/16 (88%)	16/16 (100%)
BRCA2-positive families identified	12/13 (92%)	10/13 (77%)	12/13 (92%)
Number of families with the probability >10%	67/148 (45%)	42/148 (28%)	63/148 (43%)
Mean probability for *BRCA 1/2*-carriers	53%	41%	55%
Mean probability for *BRCA 1*-carriers	50%	41%	59%
Mean probability for *BRCA 2*-carriers	55%	40%	50%
Mean probability for non-*BRCA 1/2*-carriers	12%	7%	11%

Table 3 Family history of breast and ovarian cancer of the families studied

	Total number of families	Number of mutations			
		BRCA1	*BRCA2*	non–*BRCA1/2*	Mutation %
3 affected	**74**	**6**	**2**	**66**	**10.8%**
Only breast, none under 40	47	0	1	46	2.1%
Only breast, some under 40	15	1	0	14	6.7%
Breast and ovarian, none under 40	9	3	0	6	33.3%
Breast and ovarian, some under 40	3	2	1	0	100%
4 affected	**35**	**5**	**3**	**27**	**22.9%**
Only breast, none under 40	15	0	0	15	0%
Only breast, some under 40	7	1	0	6	14.3%
Breast and ovarian, none under 40	11	3	1	7	36.4%
Breast and ovarian, some under 40	3	1	2	0	100%
>5 affected	**39**	**5**	**8**	**26**	**33.3%**
Only breast, none under 40	6	0	0	6	0%
Only breast, some under 40	10	0	2	8	20.0%
Breast and ovarian, none under 40	9	1	0	8	11.1%
Breast and ovarian, some under 40	14	4	6	4	71.4%
Total	**148**	**16**	**13**	**119**	**19.6%**
Only breast, none under 40	68	0	1	67	1.5%
Only breast, some under 40	32	2	2	28	12.5%
Breast and ovarian, none under 40	28	7	1	20	28.6%
Breast and ovarian, some under 40	20	7	9	4	80.0%

1035 unselected breast cancer patients, where all 15 cases with heavy breast cancer family history were also mutation negative (Syrjäkoski et al, 2000). Other, yet unknown susceptibility genes remain to be identified and may account for a large proportion of breast cancer families (Rebbeck et al, 1996; Serova et al, 1997; Vehmanen et al, 1997b; Ford et al, 1998; Kainu et al, 2000).

In 295 breast cancer cases with one affected 1st degree relative only one mutation (*BRCA2*, 7708 C → T) was found giving the mutation frequency of 0.3%. In this family the index patient was diagnosed at the age of 37, and her mother had died of breast cancer at the age of 40. Ovarian cancer or a young breast cancer patient diagnosed under 40 years was present in 39 families, but among these only this one mutation was found (2.6%). This suggests that mutation screening in families with only 2 affected cases is not feasible in Finland. In contrast, Goelen et al (1999) reported that *BRCA1/2* mutation testing can be done with reasonable efficiency in the Belgian population when there are 2 symptomatic family members. Prevalent founder mutations account for a large fraction of breast cancer families in Belgium (Peelen et al, 1997; Goelen et al, 1999), while *BRCA1* and *BRCA2* mutations are more rare in the Finnish population (Vehmanen et al, 1997a,b; Huusko et al, 1998). Also in studies of patients with early onset breast cancer, only a small proportion of familial risk of breast cancer has been attributed to these two genes, and the majority appears to be due to other genes (Peto et al, 1999).

CONCLUDING REMARKS

As the screening of both *BRCA1* and *BRCA2* is very laborious and expensive, and genetic testing may be emotionally very stressful for the families, the potential mutation carrier families should be recognized as efficiently as possible to avoid unnecessary analyses of non-carriers. Studies of breast cancer patients have indicated that it may be difficult to define mutation screening criteria among women with minimal or no family history (Malone et al, 1998). Furthermore, the carrier risks associated with the mutations may be highly variable, and population-based risk estimates have indicated much lower cancer risks than those obtained from multiple-case families and, therefore, lower predictive value of cancer for a positive mutation test result (Struewing et al, 1997; Fodor et al, 1998; Thorlacius et al, 1998; Hopper et al, 1999; Warner et al, 1999). Accordingly, genetic screening would be of greatest benefit in families with high cancer risk, i.e. strong family history (Fodor et al, 1998), and a high probability of harbouring a *BRCA1* or *BRCA2* mutation. For this study, we chose families with a defined family history, and developed a model by which likelihood of carrying a *BRCA1* or *BRCA2* mutation can be estimated for each family separately. It should be a useful tool in genetic counselling and focusing the mutation analyses, and increasing thus the cost-effectiveness of the genetic screening.

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