CORRESPONDENCE

BRCA phenocopies or ascertainment bias?

In a recent issue of the *Journal of Medical Genetics*, Smith *et al*[1] report a significantly higher risk of breast cancer among non-carriers in breast cancer families in which a BRCA1 or BRCA2 mutation had been identified through clinical testing. The authors found an elevated risk of approximately five-fold, which, if true, has considerable implications for the counselling and clinical management of women testing negative for the mutations found in their family.

Until the study of Smith *et al*,[1] the excess of cases observed among such non-carriers had been noted only anecdotally by many in both the clinical and research settings. In this respect, the systematic study of Smith *et al* was a welcome confirmation of these anecdotal observations. However, there are a number of methodological flaws in the analysis of Smith *et al* that render their results difficult to interpret. This stems from the fact that the families used in the analysis were not randomly sampled from the population of all potential families with a mutation, but rather had to meet certain eligibility criteria for genetic testing. Furthermore, the decision to attend a specialty oncogenetics clinic very likely depends on the family history of the individual deciding to undergo testing; the more relatives affected with breast cancer (especially diagnosed at an early age), the more likely a person is to seek genetic counselling/testing. This selection of families, whether through self-selection on family history for attending such clinics and/or through eligibility criteria for genetic testing after evaluation of family history, will result in a bias due to under-representation of families with many unaffected individuals. This bias applies to both mutation carriers and non-carriers, resulting in overestimation of both the penetrance and the phenocopy rate. Although it is possible to correct for this ascertainment bias in estimating the penetrance in carriers through conditioning on the phenotypes in the pedigree and proband genotype[2 3] it is not clear how the ascertainment issue can be properly accounted for in estimating the relative risk of disease in non-carriers.

To examine the potential magnitude of this bias, we performed the following simulation experiment. Nuclear families consisting of two parents and six offspring were simulated under a variety of phenocopy rates and penetrance values for a rare autosomal dominant disease. Each family was simulated conditional on a single affected individual who was a (heterozygous) carrier of the disease allele. This reflects the typical situation in which an affected individual is tested and found to carry the mutation and then other family members are tested for the specific mutation identified in the index case. The phenotypes (affected/healthy) and carrier status (+/–) of the other individuals in the family were simulated using the SLINK program.[4] For each set of phenocopy and penetrance values, 5000 such families were simulated in this fashion. Families were then selected for analysis according to the following ascertainment schemes:

1) No selection – all 5000 families included in analysis.
2) The probability of a family being selected in the analysis sample is linearly related to the total (including the proband) number of affected with the probabilities for 1, 2, 3, 4 and 5+ affected given by 0.0, 0.1, 0.2, 0.3 and 0.4, respectively.
3) The probability of selection in the analysis sample is more strongly related to the number of affected, with probabilities of 0.0, 0.1, 0.3, 0.6, 1.0.
4) Only families with at least two total affected (ie, the proband and at least one other) are included.
5) Only families with at least three total affected are included.

The results of these simulations are presented in table 1. It is clear that the bias in the estimate of the rate of disease in non-carriers is more strongly related to the disease risk in carriers than either the phenocopy rate or the penetrance in carriers. Although this may seem counterintuitive, the lower overall rate of disease in the families increases the effect of the ascertainment/selection. Take, for example, the case portrayed in row 2 of the table. If we select only families with at least 3 affected individuals, this eliminates all but 263 families from consideration; of these 263 families, 49 (19%) contain at least one phenocopy. In contrast, if we increase the penetrance to 0.5 and leave the phenocopy rate the same, 2900 families are included after selection on 3 affected people, of which 157 families (6%) have at least one phenocopy.

To better examine the specific BRCA testing situation as analysed by Smith *et al*, we performed additional simulations using an assumed penetrance model derived from the combined analysis of 22 studies of families of probands unselected for family history, performed by Antoniou *et al*[5] Risks were averaged over estimates for BRCA1 and BRCA2, yielding cumulative risks of breast cancer in BRCA mutation carriers of 0.10 before 40 years of age, 0.30 before 50 years, 0.44 before 60 years, 0.56 before 70 years and 0.65 until the age of 80 years. Corresponding rates in non-carriers were 0.0002, 0.002, 0.02, 0.04 and 0.06. In this case, the phenotypes of the father and two male offspring were fixed as unaffected, the mother was assumed to be aged 65 years with four daughters aged 35 (proband), 35, 45, and 45 years. In total, 10 000 such families were simulated under this model and pedigree structure.

When all 10 000 simulated families were included in the dataset, the calculated rate of disease in non-carriers was 0.0106, very close to the predicted value of 0.01105 based on the age distribution of the women in the pedigree and the risks specified above. For the linear, non-linear, 2+ and 3+ ascertainment schemes, the estimated relative risks compared with no selection were 2.76, 3.14, 2.29 and 4.74, respectively. Although we cannot know precisely which of the ascertainment models used best fits the clinical situation used to produce the data and corresponding estimate in Smith *et al*, it seems certain that their estimate is in the order of 2–3-fold too high as a result of ascertainment bias. It is noteworthy that the limited prospective data presented by Smith *et al* found a standardised incidence ratio of 2.1 (although confidence intervals were wide) which would correspond well with our estimates of the magnitude of ascertainment bias. We note that in all of the pedigrees simulated as described in the preceding paragraphs, all the phenocopies observed would correspond to type A1 phenocopies as defined by Smith *et al*[1] in their supplementary information.

Our analyses, taken together with the results of Smith *et al*,[1] still imply that women found to be non-carriers in BRCA-positive families are at perhaps twice the population risk of breast cancer, presumably due to the effects of modifier genes or correlated environmental factors. If the latter, these factors (such as parity, oral contraceptive use and menopausal status) can be adjusted for to produce individualised risk for such women. If, on the other hand, the aggregation is the result of unknown modifier loci, this cannot be done. As such loci are identified, both carrier and non-carrier women can be given better estimates of their risk.

In practical terms, there is a considerable difference between a two-fold and a five-fold increase in breast cancer risk. Doubling the risk is approximately the numerical equivalent of having a first-degree relative with post-menopausal breast cancer, which does not lead either to altered screening recommendations or generally to a recommendation for chemoprevention. A five-fold risk, on the other hand, would lead to consideration of chemoprevention in virtually all mutation-negative female first-degree relatives of mutation carriers. For example the 5-year risk for breast cancer of a 35-year-old with menarche at 11 years and first live birth at 28 years, without prior biopsy and without a family history, is 0.4% when calculated by the Gail[6] model. A five-fold increase in risk would make this otherwise low-risk woman a clear candidate for chemoprevention, with its

Table 1 Estimated relative risk of disease in non-carriers compared with simulated phenocopy rate, according to various ascertainment schemes and penetrance values

Penetrance	Phenocopy	All	Linear	Non-linear	2+	3+
0.5	0.05	1.01	1.56	1.86	1.14	1.58
0.1	0.01	1.07	3.90	4.4	3.4	6.6
0.5	0.01	1.03	1.6	1.9	1.2	1.8
1.0	0.10	0.96	1.10	1.20	0.96	0.99

associated potential toxicities. Mammographic screening and possibly even MRI would also be considered in a woman with a risk of this magnitude, with the attendant expense and high risk of false positive screens.

As Smith *et al*[1] point out, the question of whether non-carriers in families with at least one individual testing positive for a BRCA1 or BRCA2 mutation are at higher risk, and if so, what is the magnitude of this risk, can only be answered conclusively through prospective follow-up of women unaffected at the time of genetic testing. We agree with this and note that several large prospective studies of BRCA1/2 families are currently ongoing. Until the results of these studies are known, we believe that it would be premature to recommend additional screening or chemoprevention for unaffected women who test negative for the BRCA mutation segregating in their family, other than that recommended for women in their age group in the general population.

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