Major Improvement in the Efficacy of \textit{BRCA1} Mutation Screening Using Morphoclinical Features of Breast Cancer

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Abstract

A family history of breast and/or ovarian cancer is the main criterion used in screening \textit{BRCA1} gene carriers. However, ascertaining a patient’s family history is a difficult task, which significantly restricts the use of this parameter in clinical practice. Alternative individual criteria that can be used to identify \textit{BRCA1} gene carriers would, therefore, be of great value. In this context, it was recently established that \textit{BRCA1}-associated breast cancers show a specific morphoclinical pattern. In multivariate analyses, the two most discriminant morphoclinical parameters available for establishing the \textit{BRCA1} status, in addition to an early age at onset, are estrogen receptor negativity (ER−) and poor tumor differentiation (TD3). Here we tested the efficacy of these two morphological parameters as \textit{BRCA1} mutation indicators and investigated their economic impact, in a population-based survey on a series of women who developed invasive breast cancer by the age of 35 years, regardless of their family history. A high rate of 28.6% of \textit{BRCA1} mutations was found to have occurred in the group of tumors with both ER− and TD3 versus only 3.6% in tumors with other profiles (P = 0.007; odds ratio, 10.8). When the sole criterion used was early onset by the age of 35 years, the mutation rate was found to be 8.6%. The resulting cost of testing only women with ER− and TD3 tumors worked out at 30% that of testing the whole population of women with cancer by the age of 35 years, and the sensitivity was found to be of 66%. Lastly, the family history of ER− and TD3 cases with a \textit{BRCA1} mutation was investigated retrospectively, and none of these cases was found to have a particularly extensive family history of breast and/or ovarian cancer. The use of these morphological features of \textit{BRCA1}-BCs that are currently typed in clinical practice, therefore, provides a helpful and cost-effective tool for those making decisions about genetic screening. This strategy makes it possible to identify gene carriers who would be overlooked using current criteria.

Introduction

Breast carcinoma is the most common type of cancer occurring in women in the Western world. About 1 in 1,000 women develop breast cancer every year in Europe, which corresponds to almost 30,000 new cases every year in France. It is estimated that about 5% of all cases of breast cancer are transmitted as an autosomal dominant genetic trait, and a fairly high proportion of these are associated with a \textit{BRCA1} germline mutation (1). Although this proportion is small, it corresponds in fact to a large population, consisting of about 1,500 new cases every year at the French national level. Specific medical interventions need to be proposed (2) for dealing with this population because of the high penetrance of the disease, the prevalence of bilateral breast cancers, and the associated risk of developing ovarian carcinoma (1). At present, the indications for performing a \textit{BRCA1} mutation search are based on the existence of a significant family history of breast and/or ovarian carcinoma, combined or not with early age at cancer onset (3–5). However, in clinical practice, the use of these criteria is substantially restricted. Thus, based on the current indications, it has been estimated that about 500 probands (6) would be tested in France for \textit{BRCA1} mutations every year, which is far from the expected number of 1,500 patients. This limitation may be due to at least three reasons: (a) a proportion of the patients from high-risk families do not attend cancer genetic clinics; either they do not feel they are concerned or they are afraid of attending, or they are not referred to these clinics by their physicians; (b) large kindreds are becoming rare in the more highly developed countries (European reproduction rate = 1.8; Ref. 6); and, because a family’s medical history is frequently not available or has not been definitely confirmed, the anamnesis tends to be unreliable (7, 8). For all of these reasons, only 30% of the probands attending cancer genetic clinics fit the current clinical indications (6); (c) there is a lack of common family profile among \textit{BRCA1} gene carriers, and the risk of carrying a germline mutation is not limited to the members of families with a history of breast cancer (4, 9–11).

To overcome these obstacles and to improve the efficacy of genetic screening, several options may be envisaged. As far as the lack of attendance at the cancer genetic clinics is concerned, although respecting people’s right to privacy means that no pressure can be placed directly on the members of a family, it is possible to improve physicians’ knowledge about the clinical indications and DNA testing by providing specific training and information (12). However, this still leaves us with the problem of the family structure. Determining alternative individual criteria that can be used to identify \textit{BRCA1} gene carriers would certainly be extremely useful, especially if these criteria are both accessible and ascertainable and do not involve obtaining confidential medical data about relatives to establish a family’s genetic background. Interestingly, it has by now been clearly established that \textit{BRCA1}-associated breast cancers show a specific morphoclinical profile (13–16) that is potentially associated with a particular natural history (14, 17) and makes these cancers distinguishable from their sporadic counterparts as well as from other hereditary cases (18, 19). In addition, multivariate analyses were performed to select the most discriminant features among the numerous differences observed; these features, namely, estrogen receptor negativity (ER−)\(^3\) and TD3 of the tumor (20–22), could thus be taken to have a high predictive

\(^3\) The abbreviations used are: ER−, ER negativity; ER, estrogen receptor; TD, tubular differentiation; OR, odds ratio; CI, confidence interval; TD3, poor TD.
value for establishing the \( BRCA1 \) status. The expected benefit of using these parameters regardless of the family history was calculated. Although the probability of finding a \( BRCA1 \) mutation was found to be low (6%; Ref. 4) on the sole basis of early onset up to the age of 35 years, the theoretical detection rate increased considerably to between 9% and 37% when the steroid receptor negativity and differentiation status were both taken into account (20). However, this strategy still requires testing on a population-based series of cases. With a view to confirming the usefulness of morphoclinical parameters of this kind and to explore the feasibility of using them in a clinical context to orientate DNA screening procedures, we searched for \( BRCA1 \) mutations in a set of 70 consecutive cases of women affected with breast cancer by the age of 35 years without having any prior knowledge of the family history, using the ER status and the differentiation of the tumor as criteria.

**Patients and Methods**

**Patients.** To evaluate the potential value of morphoclinical parameters for \( BRCA1 \) screening, a series of almost consecutive cases of women with invasive breast cancer was constituted from the René Huguenin hospital registry. This registry contains 5700 consecutive cases of primary breast cancer in which the initial treatment was surgery, and information was available about the age at onset, ER status, and TD status, as defined previously (20). The women in whom the onset occurred by the age of 35 years amounted to 3.3% of all of the breast cancer cases. They included 70 women selected because of the availability of a biological sample (tumor and/or lymphocyte DNA) on which a \( BRCA1 \) mutation search could be performed (Table 1). In six cases, a \( BRCA1 \) disease-associated mutation (a mutation leading to a truncation of the protein, or a missense mutation in the ring finger motif; Table 2 (23)) was found. In the remaining 64 cases no proven disease-associated mutation was found in the \( BRCA1 \) gene using the current methods and conditions (24); consequently, they were considered as non-gene carrier patients. These 64 cases included 5 patients with a \( BRCA1 \) sequence variation usually taken to be a polymorphism, \textit{i.e.}, not considered as a disease-associated mutation, (3 missense mutations in the coding sequence other than those observed in the ring finger motif, and 2 cases with variations in an intronic region; Table 2), and, therefore, most of them are listed in the BIC (Breast Cancer Information Core) database\(^4\) as relatively common polymorphisms. All of the 70 cases were diagnosed between January 1980 and December 1996. Our research protocol obtained an institutional review board approval.

**\( BRCA1 \) Mutation Analysis.** Genomic DNA from women with breast cancer by the age of 35 years was prepared directly from tumor samples using a standard procedure. In addition, whenever a mutation was identified, analyses were performed on blood-cell DNA when available, prepared according to a standard procedure. To identify \( BRCA1 \) germ line mutations, DNA automated fluorescent sequencing analysis was performed on both DNA strands after denaturing gradi-

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\(^4\) Internet address: http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic.

\(^5\) Internet address: http://www.mylri.com/gtpath20.html; released on 08/12/99.
Among the five cases with *BRCA1* sequence variations corresponding to polymorphisms, none were ER− or TD3, which supports their nonpathogenic value (Table 2).

**Distribution of *BRCA1* Cases in Terms of the Family History.** The family history of the cases associated with a *BRCA1* germ line mutation and the bilateral occurrence of the breast cancer were investigated retrospectively (Table 2). Among the four ER− and TD3 cases with a *BRCA1* germ line mutation, one case (T1) did not have any family history of breast cancer, and two had a short family history, involving only one case of breast cancer in a first- or a second-degree relative (T2: the mother had breast cancer at the age of 48 years; T3: a maternal aunt had breast cancer at the age of 47 years). Only one patient had two first-degree relatives with breast cancer (T4: a sister had breast cancer at the age of 44 years, and the mother had bilateral breast cancer at the age of 37 and 42 years). None of these patients had a personal or family history of ovarian cancer. Lastly, two patients had bilateral metachronous breast cancer (T3: at the age of 29 and 41 years; T4: at 33 and 45 years).

Among the two cases with a *BRCA1* mutation and a different morphological profile, the family history of one patient (T5) had not been documented, and the remaining case had an extensive family history of breast cancer (T6: a sister with breast cancer at the age of 40 years, the mother and the grandmother with onset at an unknown age) and had bilateral breast cancer with onset at the age of 34 years and 38 years.

Among the five cases associated with *BRCA1* polymorphisms (Table 2): (a) two (T8, T11) did not have any family history of breast cancer; (b) in one (T9), the family history had not been documented; and (c) two had an irrelevant family history of breast cancer (T7: the mother with unknown age at onset; T10: a maternal aunt with unknown age at onset).

**Cost-effectiveness Benefit Ratio of *BRCA1* Testing Strategies On the basis of ER Status and Tumor Differentiation.** Assuming the overall distribution of cases in France to be that reflected in the René Huguenin hospital registry (20), about 1000 (3.3%) new cases of breast cancer are diagnosed every year by the age of 35 years in the general population. Even when there exists a true family history of cancer, there are legal obstacles to obtaining confirmation of the diagnosis from the medical records. In addition, the information about cancer risks conveyed by patients to their relatives is not always very reliable (28). On the other hand, a genetic predisposition can exist in the absence of any obvious genetic background, not only in the rare cases of proven *de novo* mutation (9), but more often in cases where the inheritance occurs via a reduced penetrance (11, 29, 30). All of these factors, in addition to the low reproduction rate of families in the Western world, significantly reduce the efficacy of the family history as the main parameter for establishing the genetic status of patients.

In addition, because of the low mutation-detection rate in the populations now under study (4, 31) using current screening methods and because of the resulting cost, performing DNA testing on the general population is not worthwhile. A *BRCA1* mutation search is usually envisaged in situations in which there is a high probability of finding a mutation, i.e., large families with a convincing history of early-onset breast and/or ovarian cancer that amount to only a small fraction of the kindreds attending cancer genetic clinics (6). However, it is of the utmost importance to make appropriate care management procedures available to potential gene carriers. This

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Breast cancer onset</th>
<th>ER status</th>
<th>TD status</th>
<th><em>BRCA1</em> mutation</th>
<th>Family history of breast cancer (yr of age)</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease-associated mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T1</strong> 34</td>
<td>ER−</td>
<td>TD3</td>
<td>ex11 g4113T/ggA→TgA ter1332</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T2</strong> 29</td>
<td>ER−</td>
<td>TD3</td>
<td>ex117 5149 delCTTA ter1678</td>
<td>Mother (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T3</strong> 29</td>
<td>ER−</td>
<td>TD3</td>
<td>ex2 185 delAG ter39</td>
<td>Mother (37, 42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T4</strong> 33</td>
<td>ER−</td>
<td>TD3</td>
<td>ex11 3957 delCTC ter1280</td>
<td>Sister (44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T5</strong> 35</td>
<td>ER+</td>
<td>TD2*</td>
<td>ex11 g3867T/gAg→TAg ter 1250</td>
<td>Sister (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T6</strong> 34</td>
<td>ER+</td>
<td>TD1</td>
<td>ex20 5382 insC ter1829</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sequence variations |
| **T7** 33 | ER+ | TD2 | ins20+23 ins12 | Mother* |
| **T8** 35 | ER+ | TD2 | ins20+23 ins12 | None |
| **T9** 34 | ER+ | TD2 | ex11 g3143A Met1008Ile | Unknown |
| **T10** 34 | ER+ | TD2 | ex15 g965T Ser1512Ile | Maternal aunt* |
| **T11** 35 | ER+ | TD2 | ex11 A14185g Arg1347Gly | None |

*BRCA1* analysis performed in both tumor and lymphocyte DNA. Patients who developed a metachronous bilateral breast cancer. *d* TD2, occasional or medium TD; TD1, throughout or high TD. *e* Age at onset unknown.
means that new parameters are needed to help to identify those with a high risk of developing breast and/or ovarian carcinoma. In this connection, it was recently predicted that individual morphological parameters might prove to be useful tools of establishing the BRCA1 status. In a recent study, we established that the differentiation and ER status of tumors are efficient indicators for identifying BRCA1 gene carriers (20). In the present study, we tested the efficacy and the feasibility of this strategy. A low overall mutation rate of 8.6% was detected in our sample of women with invasive breast cancer by the age of 35 years; whereas, 2.86% of the women with a tumor that was both ER– and TD3 had a BRCA1 germ line mutation, regardless of the family history. For the sake of comparison and to show the validity of our strategy, this observed mutation detection rate can be said to lie in between that obtained up to now when there was a family history of breast cancer only (7–18%; Refs. 24, 31, 32) and when there was a family history of both breast and ovarian cancer (33–67%; Refs. 24, 31, 32).

Other features, such as the syncytial growth pattern (16, 19) or the level of the estrogen responsive gene pS2 expression (22), may be valid indicators of BRCA1-associated breast cancer, but these parameters are not always routinely explored and/or they require the intervention of highly trained pathologists. Contrary to the method applied in other analyses (16, 19), our method relies only on the use of common parameters that are easily accessible and currently typed in clinical practice. Thus, the ER and differentiation status of tumors are highly informative parameters that are currently used to establish the prognosis of the disease and to select the appropriate initial therapeutic strategy. Knowledge of these parameters should also be useful to those making decisions about DNA testing in the context of hereditary breast cancer, particularly when the family history is either uncertified, unknown, or on a small scale. Indeed, among the four ER– and TD3 cases associated in the present study with a BRCA1 mutation, one woman without any family history of breast cancer would certainly have escaped analysis based on the previous criteria. The advisability of testing two others patients with only one first- or second-degree relative with breast cancer would have been discussed. And, finally, only one of the four cases with two affected first-degree relatives would have been tested at most of the laboratories involved in BRCA1 screening. The use of our strategy would, therefore, make it possible to identify women gene carriers who would have been overlooked if pedigree information was the sole factor taken into consideration, thus barring these women, as well as their apparently unaffected relatives, from appropriate medical interventions. Conversely, morphological features could also be helpful in establishing the nonpathogenetic value of a sequence variation of BRCA1, together with its nonsegregation among affected family members, as well as the site of the sequence variation within the BRCA1 gene (nonconserved region, outside a functional motif). In our sample, none of the five tumors associated with BRCA1 sequence variations considered as polymorphisms were both ER– and TD3 nor did any of them harbor either of these features.

From the economic point of view, the use of morphological parameters is a cost-effective strategy. The cost of testing only women with a tumor that is both ER– and TD3 would amount to only 30% of the cost of testing the whole population of women with cancer by the age of 35 years, and the sensitivity of the method is 66%, which is satisfactory.

In conclusion, it can be expected that the benefits of using morphological parameters in BRCA1 screening will not be limited to women with breast cancer by the age of 35 years because this strategy could be extended to other age groups (20), in which it could also be used together with the family history, however slight this history might be.

Acknowledgments

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