Bar code screening on combed DNA for large rearrangements of the BRCA1 and BRCA2 genes in French breast cancer families


METHODS
From January 1991 to December 1999, women with breast cancer (index cases), ascertained in the Institut Curie cancer genetic clinic were referred to our laboratory for BRCA1/2 analysis. Familial criteria for genetic testing were: at least three cases of breast cancer at any age in the same lineage. Each of the index cases was an affected family member. The probability of being a carrier of a breast cancer predisposing allele (whatever the gene involved) was obtained by using the MLINK program of the LINKAGE package with the parameters of the Claus segregation model modified by Easton, as previously described. In order to detect such rearrangements, we have developed a bar code on combed DNA for the BRCA2 gene, which leads to a panoramic view of this gene and its flanking regions, as previously described for BRCA1. We have concurrently used BRCA1 and BRCA2 bar codes to analyse a series of 26 highly selected French patients with a family history of breast cancer only, who were previously found to be negative for point or small mutations in both BRCA1 and BRCA2 genes.

RESULTS
Twenty-six unrelated French cases with a personal and family history of breast cancer (three to eight cases per family), and with at least a 90% predisposition probability (90-98%) were selected for BRCA1 and BRCA2 bar code analyses as (1) they were previously negatively tested for BRCA1/2 point or small mutations and (2) a lymphoblastoid cell line was available (table 1).

In this series of breast cancer cases, microscopic analysis of ICI421 DNA showed BRCA1 signals without LR9-12 and with half of the LR13-15 probes (fig 1). This deletion resembled the previously reported 23.8 kb deletion of exons 8-13 of family IC568. The use of primers specifically designed for genetic testing of IC568 family members detected an identical 923 bp PCR product in patient ICI421, suggesting that these two deletions resulted from a common mutation event in these two unrelated families. This was confirmed by the characterisation of a common haplotype associated with the carrier status in the two families, namely tel - D17S1327, 131 bp - D17S1323, deleted - D17S1322, 121 bp - D17S855, 141 bp - D17S1185, 216 bp - cen (data not shown). In order to examine

Key points
- In order to estimate the number of large rearrangements in the BRCA1 and BRCA2 genes, a series of 26 highly selected French patients with a family history of breast cancer only was analysed using BRCA1 and BRCA2 bar codes on combed DNA.
- One BRCA1 deletion was detected, whereas no BRCA2 rearrangement was identified.
- This study confirms the low frequency of BRCA2 rearrangements in breast cancer families.

LETTER TO JMG

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I dentification of the BRCA1 and BRCA2 genes was a major advance in the understanding of the familial forms of breast cancer, as alterations of these genes result in a high predisposition to breast cancer. To date, analysis of BRCA1 and BRCA2 coding sequences by mutation screening methods based on PCR sequencing protocols has allowed the identification of at least 900 different point or small disease causing germline alterations (Breast Cancer Information Core, BIC database). Furthermore, several large BRCA1 rearrangements have been reported, detected by Southern blotting, lymphocyte transcript analysis, or long range PCR. The majority of characterised rearrangements result from unequal recombination events between Alu sequences. We have estimated that the contribution of large rearrangements to the spectrum of BRCA1 mutations is close to 10% in French breast-ovarian cancer families. Only two rearrangements of the BRCA2 gene have been detected to date: a 5 kb deletion skipping exon 3 and a 6.2 kb deletion removing exons 12-13. However, few groups have systematically looked for BRCA2 rearrangements in defined series of breast cancer cases.

In order to detect such rearrangements, we have developed a bar code on combed DNA for the BRCA2 gene, which leads to a panoramic view of this gene and its flanking regions, as previously described for BRCA1. We have concurrently used BRCA1 and BRCA2 bar codes to analyse a series of 26 highly selected French patients with a family history of breast cancer only, who were previously found to be negative for point or small mutations in both BRCA1 and BRCA2 genes.

Identifica
the frequency of this large deletion among breast cancer only families, we screened for the presence of the specific 923 bp PCR product a series of 245 women with breast cancer, ascertained at the Institut Curie cancer genetic clinic according to previously reported family criteria and negatively tested for BRCA1 and BRCA2 point mutations. No 923 bp fragment was observed, suggesting that this large deletion is not frequent in the study population (data not shown). No other rearrangement of the BRCA1 gene was detected by either qualitative or quantitative analysis.

Table 1

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<th>IC family</th>
<th>Predisposition probability (%)</th>
<th>Age at diagnosis (y) of breast tumour for index cases</th>
<th>No of breast tumours in the family</th>
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*Three of the 26 families comprised a male breast cancer. Predisposition probabilities of index cases range from 90 to 98% (average 92%). The number of breast tumours in the family varies from 3 to 8 (average 4.5).

Figure 1

BRCA1 bar code. (A) The BRCA1 region in 17q21. The BRCA1 gene, composed of 24 exons (22 coding exons), is spread over 81 kb and has a common promoter with the NBR2 gene [Next to BRCA1 gene 2]. The BRCA1 pseudogene (ΨBRCA1) lies next to NBR2, and corresponds to a partial duplication of BRCA1, from the promoter region to intron 2. The NBR1 gene is located 5′ to ΨBRCA1. The NBR1 region is not drawn to scale and is adapted from Puget et al., Barker et al., and Brown et al. (B) Analysis of DNA from patient IC1421 with the BRCA1 bar code. (N) This full signal corresponds to the normal allele. The PAC 103014 insert covers the region between the first exons of NBR1 and the 3′ BRCA1 UTR (exon 24), which is approximately 120 kb long. The cosmid ICRF1050D6121 clone used in our experiment is rearranged, with an insert which is approximately 6 kb long and covers the region between NBR2 intron 1 and BRCA1 intron 2. The other probes are long range (LR) PCR products, covering the region between the exons quoted, except LR24-3′, which covers BRCA1 exon 24 to 10 kb downstream. (del) This full signal lacks LR9-12 and half of the LR13-15 probes, which represents a first indication of a deletion of at least exons 9 to 13 in the BRCA1 gene in this patient.
The BRCA2 gene sequence is present in PACs 214K23 and 92M18 corresponding to exons 1-24 and exons 25-27 respectively (Genbank accession numbers Z74739 and Z73359). In the “Human BAC End” database (The Sanger Centre), BAC 486017 was found to comprise both 5’ and 3’ ends of the BRCA2 gene, spreading over 84 kb. Intron sizes were precisely determined based on available data on sequences of both BRCA2 exons and PACs. Long range (LR) PCR primers were designed to obtain probes for the BRCA2 bar code (available on the Institut Curie web site), varying from 3.2 to 9 kb and spreading over several exons. This bar code is slightly different from the BRCA1 bar code, since all but two of the exons (exons 10 and 11, owing to their large sizes) are included in LR probes, and large introns are covered by the green BAC 486017 (fig 2). A total of eight probes designed the bar code, splitting the BRCA2 gene into 13 fragments. The series of 26 breast cancer cases was analysed with the BRCA2 bar code (among 46 signals captured after screening of the slide). A total of eight probes designed the bar code, splitting the BRCA2 gene into 13 fragments.

DISCUSSION

The aim of the present study was to estimate the number of rearrangements in the BRCA1 and BRCA2 genes in a series of highly selected French breast cancer families. A set of 26 cases defined by an at least 90% predisposition probability (that is, an average of 4.5 breast tumours per family), absence of a family history of breast-ovarian cancer history were analysed and only one family (owing to founder effects, since two deletions account for 36% of the alterations). The lower contribution of rearrangements to the BRCA2 mutation spectrum as compared to BRCA1 may be explained by two hypotheses: (1) the longer coding sequence (10,257 v 5592 bp) split into about 20 exons (26 v 22) and encompassing similar genomic sequence sizes (84 v 81 kb) and (2) the lower intronic density of Alu sequences (20 v 41.5%) which are the most frequently repetitive elements involved in the appearance of large gene rearrangements.

Despite complete screening for point mutations and large rearrangements in both BRCA1 and BRCA2, only one family among the 26 highly selected families of this series was explained by the identification of a BRCA1 alteration. Therefore, 25 families including three with male breast cancer still remained unexplained. This is striking as the contribution of BRCA1/2 mutations, estimated from linkage analyses, is 65% and 96% in breast only cancer families and in families with at least one male breast cancer, respectively. The failure to detect mutations may be the result of mutations not detected by the methods used, especially mutations located in introns and far from splice sites. Index cases may also be sporadic cases occurring in families with a segregating predisposing mutation. Finally, such large families with a high predisposition probability may be the result of a single gene with a recessive transmission mode or more likely additional gene variations with multiplicative effects. Considerable work remains to be done to explain breast cancer families with no BRCA1/2 mutation.

The search for BRCA1 rearrangements may now be systematically included in genetic testing. However, because of the lack of evidence for a significant contribution of BRCA2

Figure 2. BRCA2 bar code. (A) The BRCA2 gene in 13q12-13 (not drawn to scale). The BRCA2 gene is composed of 27 exons (26 coding exons) and is spread over 84 kb. Introns range in size from 91 bp (intron 5) to 14.5 kb (intron 24). (B) Probes used for the design of the BRCA2 bar code. They are presented in the colour of their revelation step (five layers of antibodies): BAC 486017 insert (approximately 180 kb long, The Sanger Centre) covers the entire region, the other probes are long range (LR) PCR products, ranging in size from 3.2 to 9 kb, covering several exons and generated from the BAC. Owing to the 5 kb length of exon 11, probe LR11-13 covers only from the 3’ end of exon 11 to exon 13. (C) Control DNA: two full signals showing the BRCA2 bar code (among 46 signals captured after screening of the slide). A total of eight probes designed the bar code, splitting the BRCA2 gene into 13 fragments.
rearrangements, it does not seem justified to include the search for them in routine genetic testing at the present time. Additional studies are needed to define clearly their role in breast cancer genetic testing.

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REFERENCES


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