Clinical Chemistry 52:8
1480–1485 (2006)

Molecular Diagnostics and Genetics

Genomic Rearrangements at the *BRCA1* Locus in Spanish Families with Breast/Ovarian Cancer

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Background: **Large genomic rearrangements (LGRs) account for a substantial proportion of the *BRCA1* disease-causing changes, or variations, identified in families with hereditary breast/ovarian cancer [HB(O)C]. Great differences in the spectrum and prevalence of *BRCA1* LGR have been observed among populations. Here we report the first comprehensive analysis of *BRCA1* LGRs conducted in Spain.**

Methods: **We used multiplex ligation-dependent probe amplification (MLPA) to screen for *BRCA1* LGRs in the index case individuals of 384 HB(O)C families who previously tested negative for *BRCA1* and *BRCA2* point variations, small insertions, and deletions. An alternative set of MLPA probes, long-range PCR, and real-time PCR were used to confirm positive results.**

Results: **We have identified 8 different *BRCA1* rearrangements (del exon 1–24, del exon 8–13, del exon 11–15, del exon 14, dup exon 19–20, dup exon 20, exon 21–22 amplification, and del exon 23–24). With the exception of del exon 8–13, they are novel alterations. Overall, *BRCA1* LGRs explain 1.4% of the Spanish HB(O)C families, and they account for 8.2% of all *BRCA1* pathogenic variations identified in our study population. *BRCA1* genetic variants affecting hybridization of commercially available MLPA probes are very rare in our population.**

Conclusions: **Screening for *BRCA1* LGRs should be mandatory in Spanish HB(O)C families. A high proportion of country-specific rearrangements are scattered along the gene. MLPA is a robust method to screen for LGRs in our population. MLPA analysis of positive samples with an alternative set of probes, together with long-range PCR and real-time PCR, is a feasible approach to confirm results in cases in which LGR breakpoints have not been characterized.**

BRCA1 (OMIM No 113705)[9] was the first breast cancer susceptibility gene identified. Several studies have reported the presence of *BRCA1* germ-line disease-causing changes, or variations, in breast/ovarian cancer families from different ethnicities and geographical areas of the world. The Spanish Consortium for Hereditary Breast and Ovarian Cancer performed a comprehensive analysis of *BRCA1* germ-line variations in Spanish families with breast/ovarian cancer. As reported in many other populations, the highest frequency of variations was found in families with both breast and ovarian cancer cases (52.1%). The frequency was much lower in site-specific families with breast cancer (15.4%). Relevant findings of that study were the high proportion of variations that appear to be unique to Spaniards (42%) and the existence

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Received March 13, 2006; accepted May 31, 2006.
Previously published online at DOI: 10.1373/clinchem.2006.070110

[9] Human genes: *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset.

of recurrent variations associated with the geographical origin of the families *(1)*.

In all populations tested, the observed frequency of *BRCA1* variations in high-risk breast/ovarian cancer families was consistently lower than predicted by linkage analysis *(2)*. This finding suggests that methods for scanning variations fail to detect all *BRCA1* germ-line defects. For instance, large genomic rearrangements (LGRs)[10] are not detectable by current PCR-based methods, perhaps explaining the discrepancy between linkage analysis and genetic testing. In support of this view, it has been proposed that the genetic structure of *BRCA1*, with numerous intragenic *alu* repeats *(3)* and 1 *BRCA1* pseudogene 30 kb upstream *(4, 5)*, might render this locus particularly prone to LGRs. Indeed, different studies have demonstrated the existence of germ-line LGRs involving either *alu* repeats *(6–10)* or the *BRCA1* pseudogene *(11)*. At least one LGR has been reported in which neither *alu* repeats nor the *BRCA1* pseudogene were involved *(12)*.

Although LGRs do not fully explain the observed discrepancy between *BRCA1* linkage analysis and variation screening, they account for a substantial proportion of *BRCA1* variations in tested populations. Unfortunately, the detection of heterozygous LGRs remains challenging *(13)*. The analysis may become straightforward in certain populations in which strong founder effects are present. For instance, 2 *alu*-mediated deletions, involving exons 13 and 22, account for a substantial proportion of all *BRCA1* variations found in Dutch families with high risk for breast/ovarian cancer *(6)* but are not present in other populations. Several approaches have been used to detect LGRs in the *BRCA1* locus, including Southern blot *(6–8)*, long-range PCR *(12)*, fluorescence in situ hybridization-based methods *(14)*, and real-time PCR *(15)*, but studies directly comparing the detection limit of those techniques are not available.

In recent years, multiplex ligation-dependent probe amplification (MLPA) has emerged as a powerful method to screen LGRs in a large number of samples *(16–18)*. We investigated the clinical relevance of the results of the Spanish Consortium for Hereditary Breast and Ovarian Cancer analysis of *BRCA1* LGRs in the Spanish population and tested the feasibility of MLPA for scanning for *BRCA1* LGRs in a genetic diagnostic laboratory.

[10] Nonstandard abbreviations: LGRs, large genomic rearrangements; MLPA, multiplex ligation-dependent probe amplification; HB(O)C, hereditary breast and/or ovarian cancer; HCSC, Hospital Clínico San Carlos in Madrid; HSP, Hospital de la Santa Creu i Sant Pau in Barcelona; IBGM, Instituto de Biología y Genética Molecular-Universidad de Valladolid in Valladolid; CNIO, Centro Nacional de Investigaciones Oncológicas in Madrid; UMM-FPGMX, Unidad de Medicina Molecular in Santiago de Compostela; CIC-US, Centro de Investigaciones del Cáncer-Universidad de Salamanca in Salamanca; ICO, Institut Català d'Oncologia in Barcelona; HBOC, breast and ovarian cancer families; HBC, breast cancer families.

Materials and Methods

Hereditary breast and/or ovarian cancer HB(O)C families—those families in which a minimum of 3 patients with breast and/or ovarian cancer were present in 2 generations of the same parental branch—were identified through family cancer services throughout Spain. Informed consent was obtained from all study participants. The contributing centers were Hospital Clínico San Carlos in Madrid (HCSC), Hospital de la Santa Creu i Sant Pau in Barcelona (HSP), Instituto de Biología y Genética Molecular-Universidad de Valladolid in Valladolid (IBGM), Centro Nacional de Investigaciones Oncológicas in Madrid (CNIO), Unidad de Medicina Molecular in Santiago de Compostela (UMM-FPGMX), Centro de Investigaciones del Cáncer-Universidad de Salamanca in Salamanca (CIC-US), and Institut Català d'Oncologia in Barcelona (ICO).

Before LGRs analysis, all families were tested for the presence of point variations at the *BRCA1* and *BRCA2* genes. In each family, variation scanning, including the full coding sequence and intron/exon boundaries of both genes, was carried out in the index case (usually the youngest affected member). Methods have been described previously *(1)* and are available on request.

A consecutive series of 417 HB(O)C families were identified during the period 1998–2004 through the HCSC, HSP, and IBGM centers. After conventional variation scanning, 67 *BRCA1*- and 65 *BRCA2*-related families were identified, and 285 families remained negative. Of this negative group, 55 were breast and ovarian cancer families (HBOC) and 230 were site-specific breast cancer families (HBC). The study performed in these families is referred to throughout the text as study I.

Other participant centers tested only those negative HB(O)C families with additional features of hereditary cancer, such as bilateral cases, concomitant breast and ovarian cancer in one patient, or very early age at diagnosis (<35 years). A total of 99 such families (32 HBOC and 67 HBC families) were tested. The study is referred to throughout the text as study II.

All participating centers used MLPA amplification to detect LGRs at the *BRCA1* locus. MLPA was performed with the *BRCA1* P002 probe mix assay according to the instructions provided by the manufacturer (MRC Holland). In all positive samples, additional analysis with the alternative set of probes P087 (MRC Holland) was performed to confirm results. Separation and relative quantification of picks was obtained with ABI-310 or ABI-3100 genetic analyzers (Applied Biosystems). Variations in peak areas were evaluated by cumulative comparison of samples from the same experiment by GeneScan software (Applied Biosystems). For statistical analysis, the individual peak areas were transferred into an Excel data sheet, and the protocols for the final assessment of allele dosage (calculating the relative peak areas) were used as described by the manufacturer (www.mrc-holland.com).

Genomic amplifications were confirmed by real-time

quantitative PCR in a LightCycler (Roche). All primers are available on request. Reactions were performed in 10 μL final volume with LightCycler-FastStart DNA Master SYBR Green I (Roche) and uracil DNA glycosilase (0.25 U). Dilutions of a control DNA were used to construct calibration curves for exons 10,19,20,21, and 22. Exon 10 was used as reference of wild-type allele dosage. Quantification of the suspected amplified exons relative to the reference exon was performed in positive samples and in control DNA by comparing crossing points with Light Cycler software versións 3.5 (Roche).

Deletions were confirmed by long-range PCR performed with LA PCR assay v2 (Takara Bio Inc), according to the supplier instructions, followed by restriction-fragment analysis.

Results

In addition to the 67 *BRCA1*-related families previously detected by conventional scanning methods, 6 families with LGRs at the *BRCA1* locus were identified in study I. *BRCA1* LGRs account for 1.4% of unselected HB(O)C families (6/417), 2.1% of unselected HB(O)C families without detectable point variations in *BRCA1* and *BRCA2* (6/285), and 8.2% of all *BRCA1* pathogenic variations identified in study I (6/73). Five additional families with *BRCA1* LGRs were identified in study II.

The prevalence of *BRCA1* LGRs according to clinical criteria is shown in Table 1. Overall, we found a higher proportion of rearrangements in HBOC families (7/87) than in HBC families (4/297). The finding was statistically significant (8% vs 1.3%; odds ratio = 6.41; 95% confidence interval, 1.63–26.8, $P = 0.0037$). The spectrum of alterations is outlined in Table 2. We have identified 8 different *BRCA1* LGRs. One of these, del exon 8–13, has been reported previously in a French family *(8)*. The remaining 7 are novel alterations not reported in other populations.

Different strategies were used to characterize the breakpoints of internal deletions. The index case of family CNIO-115 had an MLPA profile suggestive of a deletion comprising exons 8–13. A *BRCA1* deletion of exons 8–13 had been described previously in a French family *(8)*. We used specific PCR primers *(8)* to confirm that our family carried the same rearrangement, encompassing nucleotides 26967 to 50729 inclusive (GenBank accession no. L78833).

Family HCSC 221 had an MLPA profile suggestive of an exon 14 deletion in 2 affected women. One of the women had breast cancer, diagnosed at 32 years, the other had ovarian cancer, diagnosed at 42 years. No other family member was participating in the study. For both women, we performed a long-range PCR to amplify the *BRCA1* gene from exon 13 to exon 15. A fragment of ~3 kb was amplified in DNA from both affected women. The expected 8.2-kb fragment was not observed, probably because of preferential amplification of the shorter fragment. However, the 8.2-kb fragment was amplified in control DNA. Digestion of the specific 3-kb PCR product by different restriction enzymes showed that the deletion breakpoints comprised a Bgl II fragment of 6715 bp (data not shown). Different sets of primers were designed in this region. We finally designed a forward primer annealing in intron 13 (TTCTGGAGATCTGTAACTG) and a reverse primer annealing in intron 14 (TATGTTAGAATCAGATCTC), which amplified a 750-bp fragment containing the breakpoints. After sequencing, the deletion was shown to encompass nucleotides 47840 to 52790. The breakpoints involved *alu* sequences, because it has been reported for other *BRCA1* deletions *(19)*. Family CIC-US1 has an MLPA profile suggestive of an exon 14 deletion. A PCR with primers intron 13fw and intron 14rev confirmed that this family carried the same rearrangement.

The index case of family IBGM 211 showed an MLPA suggestive of a deletion spanning exons 11 to 15. A PCR was performed, with a forward primer annealing in intron10 (ctgcatacatgtaactag) and a reverse primer annealing in intron 16 (agtcattagggagatacata). In control individuals, no product was generated (the expected fragment of 25.1 kb is probably too long to be amplified in our conditions), but a 2-kb fragment was generated in this patient, supporting the presence of an internal deletion spanning ~23 kb. Both strands of the 2-kb fragment were sequenced by primer walking. The forward sequence was compatible with a deletion encompassing nucleotides 33450 to 56562 (GenBank accession no. L78833). Because of the presence of repetitive sequences, breakpoints could not be confirmed by reverse sequencing.

We have not been able to characterize the breakpoints in the remaining cases. However, we have obtained additional evidence supporting the presence of a rearrangement. The index case of family HCSC 159 had an MLPA profile compatible with a deletion encompassing the last 2 exons of the gene (23 and 24). Nine female relatives were tested. The abnormal profile was detected in all affected women (2 each of breast and 2 ovarian cancer) but in only 1 woman (age 28 years) of 5 healthy women tested. We performed long-range PCR with a forward primer located in exon 22 (tgattttacatctaaatgtc) and a reverse primer located 9.4 kb downstream of the stop codon (aagtgaagcggtgatttgctctc). The expected 13.1-kb fragment was not amplified in control DNA, but an ~6-kb fragment was consistently observed in DNA with an abnormal MLPA profile. Further analysis with

Table 1. *BRCA1* LGR in families previously tested negative for *BRCA1/2* variations.

Ascertainment	Phenotype	n	*BRCA1* LGR	Frequency, %
Study I	HBOC	55	4	7.2
	HBC total	230	2	0.9
		285	6	2.1
Study II	HBOC	32	3	9.3
	HBC total	67	2	3.0
		99	5	5.1

Table 2. Germ-line BRCA1 genomic rearrangements found in Spanish families with HB(O)C.

Family ID	Study	MLPA[a]	Segregation with disease[b]	Molecular confirmation[b]	Mapped breakpoints[c]	Phenotype	Reported in other populations[d]
HSP 198	I	Del Ex1–24	Yes		nd	HBOC	no
CNIO-115	II	Del Ex8–13	nd	Sequencing	26967–50729	HBOC	yes
IBGM 211	I	Del Ex11–15	nd	Sequencing	33450–56562[b]	HBOC	no
HCSC 221	I	Del Ex14	Yes	Sequencing	47840–52790	HBOC	no
CIC-US 1	II	Del Ex14	Yes	Sequencing	47840–52790	HBOC	no
CIC-US 2	II	Dup Ex19–20	Yes	Real-time PCR	nd	HBC	no
CIC-US 3	II	Dup Ex20	Yes	Real-time PCR	nd	HBC	no
CIC-US 4	II	Dup Ex20	Yes	Real-time PCR	nd	HBOC	no
IBGM 98	I	Ex21–22 Amplification	nd	Real-time PCR	nd	HBC	no
IBGM 107	I	Ex21–22 Amplification	nd	Real-time PCR	nd	HBC	no
HCSC 159	I	Del Ex23–24	Yes	Long-range PCR	nd	HBOC	no

nd, not determined.
[a] Probe set P002 and Probe set P087.
[b] 2 See text for further details.
[c] 3 GenBank accession no. L78833.
[d] 4 Bibliographic search December 2005.

restriction enzymes showed that an XmaI-HindIII fragment of 7.6 kb, encompassing exons 23 and 24, was lost.

In both IBGM 98 and IBGM 107 families, the index case showed an MLPA profile compatible with amplification of exons 21 and 22. In family IBGM 98, the index case showed an allele dosage of 2.84 and 2.65 for exons 21 and 22, respectively. The index case of family IBGM107 showed an allele dosage of 2.72 and 2.77. The data were compatible with the presence of 3 or 4 extra copies of both exons in the families. Additional analysis by real-time quantitative PCR indicated the presence of a minimum of 4 extra copies of both exons, but neither the precise number of extra copies nor the size of the amplicon has been fully characterized, and we cannot rule out the possibility that the families carried different rearrangements.

Similarly, MLPA allele dosage and real-time quantitative PCR confirmed the presence of 1 extra copy of exons 19 and 20 in family CIC-US 2, and 1 extra copy of exon 20 in families CIC-US 3 and CIC-US 4.

The index case of family HSP-198 showed an MLPA profile suggestive of a deletion involving the complete *BRCA1* gene. This alteration was confirmed to segregate with the disease in the family. It was detected in 4 female relatives; 1 with ovarian cancer case diagnosed at 41 years, 1 with breast cancer diagnosed at 43 years, 1 with endometrial cancer diagnosed at 41 years, and 1 healthy woman at 45 years. The alteration was not present in a 50-year-old healthy woman.

Finally, it is worth mentioning that only ~1 of 348 samples tested with the P002 probe set carried a DNA variant hampering probe hybridization. The *BRCA1* variant 5214 C to T, a missense variation (R1699W) of uncertain pathogenic significance, is located at the ligation site of the exon 18 probe (U14680, 5213–5214). MLPA analysis with the alternative P087 probe set, which has a ligation site 41 nucleotides downstream (U14680, 5254–5255) discarded the presence of an exon 18 deletion.

Discussion

We conducted independent analysis in 2 family series selected with different criteria. Following the guidelines of our Consortium, HB(O)C families with a minimum of 3 breast and/or ovarian cancer cases diagnosed in 2 generations of 1 parental branch are eligible for genetic testing, irrespectively of the age at diagnosis or the presence of additional features of hereditary cancer. Study I was conducted in a consecutive series to estimate the actual prevalence of LGRs in such families. Study II was performed in a nonconsecutive group of HB(O)C families with additional features of hereditary cancer. The latter study was conducted in those laboratories in which cost-effectiveness was a priority.

Study I showed a 2.1% prevalence of BRCA1 LGRs in Spanish HB(O)C families previously testing negative for point variations and small insertions/deletions in *BRCA1* and *BRCA2*. A recent study performed in Australian and New Zealand families found *BRCA1* LGRs in 2.2% of the negative families *(19)*. The entry criteria in the latter study were similar to those in study I. LGRs have been reported in 6% of German *(20)*, 5.9% of French *(21)*, and 4.4% of UK *(22)* *BRCA1/2*–variation-negative families. However, without exception, these studies were performed in families with additional hereditary cancer features, such as bilateral disease or early age at diagnosis. Therefore, these studies cannot be directly compared to study I. In fact, selection criteria in these studies were similar to those in study II, in which we found LGRs in 5.1% of the negative families.

The fact that different studies report different prevalences of *BRCA1* LGRs may reflect the different genetic backgrounds of participants. This conclusion is not

straightforward, however, because other variables may explain such differences. Methodology is unlikely to be a major factor, because the same commercially available MLPA assay is used in most of the cited reports (the French study relies on a complete different approach, based on fluorescent hybridization of DNA probes on combed DNA). Study size may be relevant. For instance, the German report was conducted in 75 families, and the Australian/New Zealand study included 312. However, we think that the most likely explanation for differences is selection bias. There is no consensus on which clinical criteria should be followed in the selection of HB(O)C families. As a result, studies performed in different countries select families on the basis of different clinical criteria. The effect of selection bias (for instance, a consecutive series vs selection of families with high-risk features) on prevalence may be strong, as our own data reveal (study II compared with study I). For this reason, it is often meaningless to compare prevalence among studies.

In our view, the proportion of variations that LGRs account for in a given population is more informative. First, this rate is independent of selection bias (assuming that LGRs confer cancer risk similar to that of point variations) and can therefore be directly compared among studies. Second, it will indicate whether it may be more cost effective to perform LGR testing before or after conventional screening. According to study I, 8.2% of the *BRCA1* variations present in Spanish HB(O)C families are LGRs. This rate is very similar to that of German (8%), French (9.5%), and US (8%) populations and slightly lower than the UK (13.5%) or Australia/New Zealand (14.9%) populations *(6, 19–22)*. A higher proportion of LGRs has been found in Holland, but this is attributable to the del 13 and del 22 founder variations, which account for 24% of all *BRCA1* variations in this population *(17)*. The highest proportion of LGRs found so far was in a North Italian population (40%), although the sample size in this study was small *(17)*.

The percentage of LGRs detected in our study supports the view that screening for these variations should be mandatory in Spanish families in which a minimum of 3 breast and/or ovarian cancer patients are present in 2 generations of the same parental branch.

Both Hartman et al. *(20)* and Bunyan et al. *(22)* suggested that, in their study populations, MLPA may be marginally more cost effective as a first line of screening in the clinical setting. This may also be true in the Spanish population. However, we think that the main advantage of using MLPA as a first line is not cost effectiveness, but rather the fact that it will greatly reduce the reporting time for as much as 8.2% of the positive families. However, we should stress the importance of excluding the presence of sequence variations at the target sequences to interpreting an MLPA profile correctly.

We found 8 different *BRCA1* LGRs in our population. Of these, only 1 (del exon 8–13) has been described outside Spain. The spectrum of LGRs observed in our population has 2 interesting features. First, 2 alterations involve sequences at the 3′ end of the gene (del exon 23–24 and del exon 1–24). This finding suggests that, in addition to the high density of intragenic *alu* repeats *(2)* and the upstream *BRCA1* pseudogene *(11)*, downstream sequences may render the *BRCA1* locus particularly prone to rearrangements (in this regard, it is interesting to note that *alu* repeats are highly frequent in a 35-kb genomic region downstream of *BRCA1* exon 24). Second, we detected a considerable proportion of genomic amplifications (3 of 8), whereas most *BRCA1* LGRs identified in other populations are deletions *(22, 23)*.

Three alterations (del exon 14, dup exon 20, and exon 21–22 amplification) were observed twice in unrelated families from the Castile region. Del exon 14 was fully characterized in both families, demonstrating it to be identical at the molecular level. Dup exon 20 and exon 21–22 amplification could not be fully characterized at the molecular level, but nonetheless, the study suggests the existence of recurrent variations associated with the geographical origin of the families.

Taken together, our data indicate the presence of a high proportion of country-specific alterations and genetic diversity among subpopulations, in agreement with a previous study by our consortium showing that a high proportion of the *BRCA1* point variations found in Spain are country specific, with local founder effects *(22)*.

Our data support the view that *BRCA1* LGRs are equivalent to point variations in terms of cancer risk. First, the proportion of LGRs was higher in HBOC (8%) than in HBC (1.3%) families, indicating a strong association with ovarian cancer risk. Second, the 4 site-specific breast cancer families with LGR identified in our study had defects downstream of exon 18, supporting a tendency for families with variations toward the 3′ end of the gene to have a lower incidence of ovarian cancer *(18, 24)*.

We have not characterized the breakpoints in 5 LGRs (del exon 1–24, dup exon 19–20, dup exon 20, exon 21–22 amplification, and del exon 23–24). The molecular characterization of genomic rearrangements is time consuming and technically demanding, especially if genomic amplifications or deletions involve regions outside the *BRCA1* locus. If the molecular characterization of a suspected LGR is not feasible, genetic testing laboratories should develop alternative strategies to exclude the possibility of false positives. In the present study, all genomic rearrangements have been confirmed by an alternative set of MLPA probes (*BRCA1* probe set P087). To further exclude the presence of false positives, additional molecular evidence (long-range PCR or real-time PCR) has been obtained in all positive samples, with the exception of the sample carrying a whole-gene *BRCA1* deletion. This double-checking confirms the presence of a genomic rearrangement in those cases in which breakpoints have not been characterized.

The possibility of commonly occurring variants at or

near the probe binding site is a well-known source of MLPA false positives (as identified by the company in the assay inserts). Therefore, depending on the variants present in a given population, the screening may become troublesome. Our data indicate, however, that this is not a major concern in our population. Bearing in mind all of these considerations, we think that MLPA is a feasible method to scan for BRCA1 LGRs in a genetic diagnostic laboratory.

This work was supported by grants from Instituto de Salud Carlos III (FIS 01/3040, FIS 04/1832, FIS RTICC C03/10, and Xunta de Galicia (PGIDIT02PXIC20804PN). Sara Gutierrez-Enriquez is supported by a contract financed by Red Genómica del Cáncer del Instituto de Salud Carlos III (2003–2005). We express our appreciation to the families.

References

1. Diez O, Osorio A, Duran M, Martinez-Ferrandis JI, de la Hoya M, Salazar R, et al. Analysis of BRCA1 and BRCA2 genes in Spanish breast/ovarian cancer patients: a high proportion of mutations unique to Spain and evidence of founder effects. Hum Mutat 2003;22:301–12.
2. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 1998;62:676–89.
3. Smith TM, Lee MK, Szabo CI, Jerome N, McEuen M, Taylor M, et al. Complete genomic sequence and analysis of 117 kb of human DNA containing the gene BRCA1. Genome Res 1996;6:1029–49.
4. Barker DF, Liu X, Almeida ER. The BRCA1 and 1A1.3B promoters are parallel elements of genomic duplication at 17q21. Genomics 1996;38:215–22.
5. Brown MA, Jones KA, Nicolai H, Bonjardim M, Black D, McFarlene R, et al. Physical mapping, cloning, and identification of genes within a 500-kb region containing BRCA1. Proc Natl Acad Sci U S A 1995;92:4362–6.
6. Petrij-Bosch A, Peelen T, van Vliet M, van Eijk R, Olmer R, Drusedau M, et al. BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. Nat Genet 1997;17:341–5.
7. Swensen J, Hoffman M, Skolnick MH, Neuhausen SL. Identification of a 14 kb deletion involving the promoter region of BRCA1 in a breast cancer family. Hum Mol Genet 1997;6:1513–7.
8. Puget N, Stoppa-Lyonnet D, Sinilnikova OM, Pages S, Lynch HT, Lenoir GM, Mazoyer S. Screening for germ-line rearrangements and regulatory mutations in BRCA1 led to the identification of four new deletions. Cancer Res 1999;59:455–61.
9. Montagna M, Dalla Palma M, Menin C, Agata S, De Nicolo A, Chieco-Bianchi L, et al. Genomic rearrangements account for more than one-third of the BRCA1 mutations in northern Italian breast/ovarian cancer families. Hum Mol Genet 2003;12:1055–61.
10. Rohlfs EM, Puget N, Graham ML, Weber BL, Garber JE, Skrzynia C, et al. An Alu-mediated 7.1 kb deletion of BRCA1 exons 8 and 9 in breast and ovarian cancer families that results in alternative splicing of exon 10. Genes Chromosomes Cancer 2000;28:300–7.
11. Puget N, Gad S, Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonnet D, Lenoir GM, et al. Distinct BRCA1 rearrangements involving the BRCA1 pseudogene suggest the existence of a recombination hot spot. Am J Hum Genet 2002;70:858–65.
12. Payne SR, Newman B, King MC. Complex germline rearrangement of BRCA1 associated with breast and ovarian cancer. Genes Chromosomes Cancer 2000;29:58–62.
13. Armour JA, Barton DE, Cockburn DJ, Taylor GR. The detection of large deletions or duplications in genomic DNA. Hum Mutat 2002;20:325–37.
14. Gad S, Aurias A, Puget N, Mairal A, Schurra C, Montagna M, et al. Color bar coding the BRCA1 gene on combed DNA: a useful strategy for detecting large gene rearrangements. Genes Chromosomes Cancer 2001;31:75–84.
15. Barrois M, Bieche I, Mazoyer S, Champeme MH, Bressac-de Paillerets B, Lidereau R. Real-time PCR-based gene dosage assay for detecting BRCA1 rearrangements in breast-ovarian cancer families. Clin Genet 2004;65:131–6.
16. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification [Electronic letter]. Nucleic Acids Res 2002;15:30:e57.
17. Hogervorst FB, Nederlof PM, Gille JJ, McElgunn CJ, Grippeling M, Pruntel R, et al. Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. Cancer Res 2003;63:1449–53.
18. Montagna M, Santacatterina M, Torri A, Menin C, Zullato D, Chieco-Bianchi L, et al. Identification of a 3 kb Alu-mediated BRCA1 gene rearrangement in two breast/ovarian cancer families. Oncogene 1999;18:4160–5.
19. Woodward AM, Davis TA, Silva AG, Kirk JA, Leary JA, kConFab Investigators. Large genomic rearrangements of both BRCA2 and BRCA1 are a feature of the inherited breast/ovarian cancer phenotype in selected families. J Med Genet 2005;42:e31.
20. Hartmann C, John AL, Klaes R, Hofmann W, Bielen R, Koehler R, et al. Large BRCA1 gene deletions are found in 3% of German high-risk breast cancer families. Human Mutat 2004;24:534.
21. Gad S, Caux-Moncoutier V, Pages-Berhouet S, Gauthier-Villars M, Coupier I, Pujol P, et al. Significant contribution of large BRCA1 gene rearrangements in 120 French breast and ovarian cancer families. Oncogene 2002;21:6841–7.
22. Bunyan DJ, Eccles DM, Sillibourne J, Wilkins E, Thomas NS, Shea-Simonds J, et al. Dosage analysis of cancer predisposition genes by multiplex ligation-dependent probe amplification. Br J Cancer 2004;91:1155–9.
23. Mazoyer S. Genomic rearrangements in the BRCA1 and BRCA2 genes. Human Mutat 2005;25:415–22.
24. Thompson D, Easton D. Breast Cancer Linkage Consortium. Variation in BRCA1 risks by mutation position. Cancer Epidemiol Biomarkers Prev 2002;11:329–36.