ONLINE MUTATION REPORT

The contribution of germline rearrangements to the spectrum of *BRCA2* mutations

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Background: Few germline *BRCA2* rearrangements have been described compared with the large number of germline rearrangements reported in the *BRCA1* gene. However, some *BRCA2* rearrangements have been reported in families that included at least one case of male breast cancer.

Objective: To estimate the contribution of large genomic rearrangements to the spectrum of *BRCA2* defects.

Methods: Quantitative multiplex PCR of short fluorescent fragments (QMPSF) was used to screen the *BRCA2* gene for germline rearrangements in highly selected families. QMPSF was previously used to detect heterozygous deletions/duplications in many genes including *BRCA1* and *BRCA2*.

Results: We selected a subgroup of 194 high risk families with four or more breast cancers with an average age at diagnosis of ≤ 50 years, who were recruited through 14 genetic counselling centres in France and one centre in Switzerland. *BRCA2* mutations were detected in 18.6% (36 index cases) and *BRCA1* mutations in 12.4% (24 index cases) of these families. Of the 134 *BRCA1/2* negative index cases in this subgroup, 120 were screened for large rearrangements of *BRCA2* using QMPSF. Novel and distinct *BRCA2* deletions were detected in three families and their boundaries were determined. We found that genomic rearrangements represent 7.7% (95% confidence interval 0% to 16%) of the *BRCA2* mutation spectrum.

Conclusion: The molecular diagnosis of breast cancer predisposition should include screening for *BRCA2* rearrangements, at least in families with a high probability of *BRCA2* defects.

METHODS

Patients

Inclusion criteria for families were at least four cases of female breast cancer in the same parental lineage and average age at diagnosis of 50 years or less. Families also presenting ovarian or male breast cancer cases were included. All index cases had been found negative for *BRCA1* and *BRCA2* mutations in previous analyses, including screening for large deletions or duplications of *BRCA1*. Screening of all exons and exon-intron boundaries for point mutations and microdeletions/insertions was carried out by direct sequencing or by denaturing high performance liquid chromatography (dHPLC). All DNA samples studied were from centres with institutional and national approval to carry out molecular diagnosis of *BRCA1* and *BRCA2* defects. All patients had signed the appropriate informed consent for participation in mutation analysis.

Screening for *BRCA2* rearrangements

QMPSF was carried out as described recently.³ DNA concentrations were measured using a picogreen assay (PicoGreen dsDNA Quantitation Kit, Invitrogen, Cergy-Pontoise, France) and 100 ng samples were used in each QMPSF assay. The deletion of exons 12 and 13 found in one family was confirmed by using a multiplex ligation-dependent probe amplification (MLPA) *BRCA2* kit (MRC Holland, Amsterdam, the Netherlands). All rearrangements found were confirmed by long range PCR as previously described⁸ and the boundaries of each deletion were determined by DNA sequencing. Conditions used for long range PCR and the sequences of primers used in QMPSF assays are available upon request.

RESULTS AND DISCUSSION

We have estimated the prevalence of *BRCA1* and *BRCA2* mutations by family type, considering the number of cases as well as cancer type and age at diagnosis, in families studied in 14 genetic counselling centres in France and one centre in Switzerland and tested for mutations over a 1 year period. From subgroups with relatively large numbers of families and a high proportion of *BRCA2* mutations, we selected a subgroup consisting of 194 families with four or more breast cancers with an average age at diagnosis of 50 years or less.

BRCA2 mutations were detected in 36 index cases (18.6%) and *BRCA1* mutations, including one germline *BRCA1* rearrangement, were detected in 24 index cases (12.4%) from these families. Among the 134 *BRCA1/2* negative index cases from this subgroup, 120 were screened for large rearrangements of *BRCA2* using QMPSF.^{3, 8} The QMPSF assay covers all 27 exons of *BRCA2*, except for exon 24, which is separated from

Screening for *BRCA1* rearrangements has become part of the routine molecular diagnosis of predisposition to breast/ovarian cancer, but relatively few *BRCA2* germline rearrangements have been reported.¹⁻⁶ Germline rearrangements of *BRCA1* and *BRCA2* have been recently reviewed.⁷ We have previously shown that *BRCA2* germline rearrangements are present in a significant number of male breast cancer families³ and this finding suggested that they might represent a sizable proportion of the *BRCA2* mutation spectrum. To test this hypothesis in more depth, we screened for *BRCA2* rearrangements in 120 high risk breast cancer families negative for *BRCA1* and *BRCA2* mutations, by using quantitative multiplex PCR of short fluorescent fragments (QMPSF), a rapid and sensitive method able to detect heterozygous genomic deletions and duplications.^{3, 8, 9}

Figure 1 Detection by QMPFS of a novel deletion of *BRCA2* exons 14–18. Fluorescence profiles obtained from patient DNA and control DNA (red and blue, respectively) were superimposed and adjusted with the GeneScan (Applied Biosystems, Foster City, CA) program to match the peak heights of the control amplicon (C), consisting of a short segment of the *MLH1* gene. The deletion of five consecutive exons is shown by a ~50% reduction in the heights of the corresponding peaks.

exon 23 by a very short intron. The large exon 11 is targeted by two distinct amplicons located near its 5' and 3' ends. All QMPFS assays were carried out in the same laboratory using

as controls DNA from a chromosome 13 trisomy case and from a patient with a complete *BRCA2* deletion.³ Three families revealed distinct *BRCA2* rearrangements (fig 1 and table 1).

Table 1 <i>BRCA2</i> germline rearrangements found in this study			
Family	Number of breast cancer cases per family (age at diagnosis, years)	Average age at diagnosis, years	<i>BRCA2</i> rearrangement
IC3808	8 (32, 39, 40, 40, 40, 43, 45, 52)	41.4	del ex14–ex18
PS1188	4 (35, 46, 50, 70)	50.2	del ex15–ex16
C2309	6 (30, 58, 55, 35, 36/41, 70)	47.3	del ex12–ex13

Table 2 Summary of *BRCA2* germline rearrangements

Rearrangement	Breakpoints		Reference
Del ex 3	AY436640: [g.5029insCCAT; g.5030_10094del]	No <i>Alu</i> involved	Nordling <i>et al.</i> ¹ Mazoyer ⁷
Del ex 12–13	AY436640: [g.27631ins(A) ₆₀ ; g.27632_33852del]	<i>Alu</i> Sx/non- <i>Alu</i>	Wang <i>et al.</i> ² Mazoyer ⁷
Del ex 12–13	AY436640: g.27566_35521del	<i>Alu</i> Ya5/ <i>Alu</i> Sx	Tournier <i>et al.</i> ⁸
Del whole gene	ND*		Tournier <i>et al.</i> ⁸
Dup ex 1–2	ND*		Tournier <i>et al.</i> ⁸
Del ex 1–2	ND*		Bunyan <i>et al.</i> ⁴
Del ex 1–2†	ND*		Woodward <i>et al.</i> ⁹
Del ex 14–16	ND*		Woodward <i>et al.</i> ⁹
Del ex 17–18	AY436640: g.45138_55975del	No <i>Alu</i> involved	Agata <i>et al.</i> ⁶
Del ex 7–11	AY436640: g.12323_26644del	No <i>Alu</i> involved	Agata <i>et al.</i> ⁶
Del ex 20	AY436640: g.56447_61399del	<i>Alu</i> Sq/ <i>Alu</i> Sp	Agata <i>et al.</i> ⁶
Del ex 14–18‡	AY436640: g.37838_54065del	<i>Alu</i> Y/ <i>Alu</i> Sg	
Del ex 15–16‡	AY436640: [g.42188insTAAATACTGAC; g.42189_47824del]	Non- <i>Alu</i> / <i>Alu</i> Jo	
Del ex 12–13‡	AY436640: g.27422_36313del	<i>Alu</i> Ya5/ <i>Alu</i> Y	
Ins <i>Alu</i> ex 22	AY436640: g.65122_65123insAlu		Mazoyer, ⁷ Miki <i>et al.</i> ¹⁰
Ins <i>Alu</i> ex 3	AY436640: [g.4895_4909dup; g.4909_4910insAlu]		Teugels <i>et al.</i> ¹¹

*ND, not determined; †found twice, not known if recurrent; ‡this work.

The deletion of exons 14–18 (family IC3808) and the deletion of exons 15 and 16 (family PS1188) had not been previously described, whereas deletions of exons 12 and 13 had previously been reported in other families.^{2,3}

Long range PCR assays followed by sequencing analysis were used to determine the boundaries of each of the three deletions. In particular, it was found that the boundaries of the exon 12-exon 13 deletion differ at the sequence level from the other two reported deletions involving the same exons (table 2). While most *BRCA1* and *BRCA2* rearrangements, whose boundaries have been characterised, involve at least one *Alu* repeat, a recent report indicates that this is not always the case for *BRCA2* rearrangements, suggesting the presence of other recombinogenic elements.⁶

Two of the three novel *BRCA2* deletions found in the present study are mediated by unequal homologous recombination involving two *Alu* repeats, whereas the deletion of exons 15 and 16 reveals the presence of an *Alu* repeat only at the breakpoint within intron 16, while the other breakpoint is at the 3' boundary of intron 14 (table 2). This table shows that the boundaries of *BRCA2* rearrangements are more often within introns, and especially within *Alu* sequences. However, large deletions with boundaries in exons as well as insertions of *Alu* elements into exons of *BRCA1* or *BRCA2* have also been reported.^{1,7,10,11} This type of rearrangement may be missed in some cases by gene dosage methods, such as QMPFS or MLPA¹² which target only portions of exons or exon-intron boundaries. In this regard, it is worth noting that QMPFS (range of amplicon sizes from 152 to 280 bp) provides larger coverage of *BRCA2* exons than MLPA (target size ~60 nucleotides). While large double blinded studies are still lacking, our present experience indicates that both methods provide similar sensitivity of detection, if one compares profiles obtained from good quality DNA samples, preferably extracted with the same method. With both techniques, confirmation by a second method is necessary when deletions or duplications are indicated by a change affecting a single peak. MLPA is a multi-step assay, in which probe hybridisation, probe ligation, and PCR amplification are performed sequentially, whereas QMPFS involves only one step before capillary electrophoresis. This feature of QMPFS minimises the risk of sample cross-contamination and facilitates full automation of the assay. Conversely, MLPA allows analysis of the entire *BRCA2* gene in a single run of a capillary electrophoresis procedure, whereas, using QMPFS, three QMPFS reactions followed by three parallel capillary runs are currently needed for each DNA sample (fig 1).

The three *BRCA2* deletions identified in the present screening can be added to 36 *BRCA2* mutations previously

detected in the subgroup of 194 families selected for this study (four or more breast cancers with an average age at diagnosis of ≤50 years). Our finding suggests that 7.7% (95% confidence interval 0% to 16%) of *BRCA2* mutations are germline rearrangements. This estimate appears to be rather similar to that recently reported in a study of 121 selected breast and breast-ovarian cancer families from Italy, where three deletions were found and their contribution to the *BRCA2* spectrum was estimated to be 11% (95% confidence interval 3% to 23%).⁶ Considering the large confidence intervals, the conclusions of both studies are in good agreement and suggest that the contribution of rearrangements to the mutation spectrum of *BRCA2* is comparable to that of rearrangements in the mutation spectrum of *BRCA1*, currently estimated at about 15%.⁷

The present study extends the conclusions of our previous screening of male breast cancer families for *BRCA2* rearrangements³ and concurs with two recent reports^{5,6} in suggesting that searching for large *BRCA2* deletions/duplications should be added to the procedure for the molecular diagnosis of breast cancer predisposition, in particular in breast cancer families having a high probability that their disease is due to a *BRCA2* defect, as in families with multiple cases of female breast cancer and families with cases of male breast cancer.

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ELECTRONIC-DATABASE INFORMATION

Accession numbers (www.ncbi.nlm.nih.gov) are as follows: *BRCA2*, AY436640 (genomic); *BRCA1*, OMIM 113705; *BRCA2*, OMIM 600185

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